MYCOTOXINS:

A CANADIAN PERSPECTIVE

NRCC No. 22848

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MYCOTOXINS: A CANADIAN PERSPECTIVE

Edited by:

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Prepared for the Subcommittee
on Biological Phenomena

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PREFACE

Mycotoxins are produced by molds infesting not only plant crops in the field and during storage, but also food products destined for human consumption. Although much is known about certain mycotoxins, especially aflatoxin, there is much that we still do not know about the broad range of toxic mold metabolites, the occurrence of these metabolites in feed and food commodities, and the illnesses associated with them in animals and humans.

Issues of critical importance to the agri-food industries and regulatory agencies in Canada include:

- the impact of fungal invasion and mycotoxin formation on the production of cereal crops and other food products;
- the safety of mycotoxin-contaminated grains consumed directly by humans, as well as of mycotoxin residues in animal products;
- the significance of mycotoxin-contaminated feeds on animal health and productivity, and on the profit margins of farm producers;
- the means to prevent fungal invasion and mycotoxin production in feed and food products, and the methods of effectively detoxifying contaminated material;
- the protection of workers exposed while handling contaminated commodities or engaged in research on mycotoxins.

One of the driving forces for bringing together Canadian information on mycotoxins was the detection of deoxynivalenol (DON, vomitoxin) in the eastern Canadian winter and spring wheat crops in 1980. At that time, the lack of rapid, sensitive analytical methods and supplies of pure standards made it difficult to assess the nature and extent of the mycotoxin contamination.

Reference to "yellow rain" in this report might appear irrelevant in the Canadian context. The use of chemical warfare (CW) agents does, however, contravene multinational conventions and has implications for Canadian national security.

In response to concerns expressed by the Canadian scientific community, the Subcommittee on Biological Phenomena of the National Research Council of Canada (NRCC) Associate Committee on Scientific Criteria for Environmental Quality (ACSCEQ) appointed a panel of experts to prepare a report on the current state of knowledge regarding the mycotoxins of greatest interest to Canadians and researchers in Canada. The multidisciplinary panel was formed in 1982 and consisted of 22 scientists actively engaged in research on mycotoxins. This, their report, is not meant to be an exhaustive, detailed review, but rather a general document, summarizing what is known in the areas covered, for use by scientists initiating mycotoxin research, workers in the field and executives in industry, government and universities in Canada.

To keep the document reasonable in size, some aspects of mycotoxins have not been covered. The document does, however, deal with a broad range of interests of Canadian researchers, including descriptions of mycotoxins found in Canada or of concern to Canadian scientists, the nature and occurrence of mycotoxins in Canadian feeds and food commodities, mold infection and mycotoxin production in the field and during storage, the nutritional and toxicological effects of mycotoxins found in Canada, and the human health hazards and regulatory aspects of mycotoxin contamination. Brief descriptions of chemical methods of analysis, and requirements for reference standards, radiolabelled material and pure toxins for research purposes, as well as of chemical methods of decontamination are included. The final section, a general discussion, highlights areas of research that need to be pursued to resolve the many outstanding issues associated with mycotoxins in Canada. In the executive summary that follows, the contents of the document are briefly summarized.

For those involved in or planning studies on mycotoxins, Appendix I was prepared to provide general guidelines for the safe handling of mycotoxins.

The scientific contributions of the panel of authors and the references in this report provide a strong multidisciplinary base of scientific knowledge for those needing further information. Additional reference material can also be obtained through the "Mycotox File", maintained by Dr. A. Taylor, NRCC Atlantic Research Laboratory, Halifax, N.S. (Brewer et al. 1978), or from other scientific data bases.

EXECUTIVE SUMMARY

This document summarizes available information on mycotoxins as they affect human and farm animal populations in Canada. Mycotoxins may occur in both imported and Canadian-grown foodstuffs and feedstuffs. Among the imports of concern to the food industry and the Health Protection Branch (HPB) of Health and Welfare Canada (HWC) are peanuts, Brazil nuts and pistachios containing aflatoxins, because of the highly carcinogenic properties of aflatoxin B_1 and the potential human health hazard. Unlike the situation in certain other countries, there appears to be no problem of aflatoxin occurrence in Canadian-grown crops and feedstuffs. In addition to dicoumarol, which is not a true mycotoxin, those mycotoxins that do occur to a significant extent in Canadian commodities are Fusarium toxins, notably zearalenone and the trichothecene deoxynivalenol (DON, vomitoxin), ergot alkaloids - all formed in grains - and patulin in apple juice; to a lesser extent, ochratoxin A may also be present in Canadian grains and feeds. DON has been found mainly in wheat grown in eastern Canada. The impact of that fact on the grain trade and the concern regarding human and animal health have generated considerable Canadian research and regulatory effort on this mycotoxin.

In general, mycotoxins can be produced both in the field and during storage, although the fungi involved may be different in each case. Important genera of fungi that may produce mycotoxins on living plants and that are of relevance to the Canadian situation include Fusarium, Alternaria, Claviceps, Chaetomium, and Trichoderma. In stored products, Aspergillus and Penicillium are the major spoilage fungi and many mycotoxigenic species are known.

Only limited information is available on disease outbreaks in Canadian livestock and poultry that may be associated with mycotoxins. The difficulty in making these associations is discussed, together with symptomatology. Although livestock and poultry producers may be aware when acute mycotoxicosis occurs, the effects of chronic mycotoxicosis at low toxin levels on animal productivity, which include changes in feed consumption, weight gains, reproductive performance, egg production and resistance to infections, may go unnoticed, yet still result in economic losses. When DON was detected in winter and spring wheats in eastern Canada in 1980, extensive research with experimental animals, livestock and poultry demonstrated that concentrations up to 2 mg $DON.d^{-1}.kg\ diet^{-1}$ could be fed without serious adverse effects. Indeed, feeding trials with naturally contaminated wheat supported earlier observations that swine were very sensitive, whereas dairy cattle and poultry were more tolerant. At the DON concentrations detected in eastern wheat, the mycotoxin should not pose a serious problem to farm animals, except possibly swine. Research has been carried out in Canada demonstrating that in some cases dietary supplements may be used in the prevention and treatment of mycotoxicosis. Decontamination of feedstuffs is not currently practiced in Canada, although some Canadian research on

the destruction of aflatoxins and DON has been carried out in addition to studies on the effects of milling and baking on DON distribution and retention.

At present no known human toxicoses of Canadians can be attributed to the presence of any mycotoxins in the diet. In some African and Asian countries, however, an association of dietary aflatoxin exposure and a high incidence of primary liver cancer has been demonstrated. In Canada the exposure to aflatoxin and the incidence of liver cancer are among the lowest in the world. Canadian regulations for aflatoxins and DON in foods and foodstuffs have been established, and adequate methodology has been developed for these and several other mycotoxins.

There are many mycotoxin issues in Canada that require intensive research:

- well designed surveys on the nature and extent of toxigenic fungi in Canada and mycotoxins in feed and foodstuffs, and associated illness and poor productivity in livestock
- studies on factors related to mold infection and toxin production in the field and in storage
- methods to decontaminate or detoxify animal feeds and food products
- research on the nutritional, pharmacologic and toxicologic effects of mycotoxins, including mycotoxin interactions
- assessment of health hazards of personnel handling mycotoxin-contaminated material
- development of rapid, sensitive analytical methods, pure mycotoxin standards and radiolabelled mycotoxins

MYCOTOXINS - A CANADIAN PERSPECTIVE

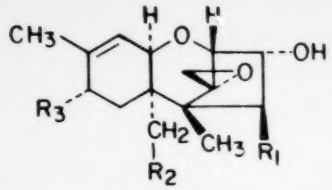
1.0 BACKGROUND

1.1 MYCOTOXINS (P.M. Scott)

Mycotoxins may be defined as secondary metabolites produced by filamentous fungi and having deleterious biological effects in animals and/or man. Important genera of fungi that include mycotoxin-producing species are Aspergillus, Penicillium, Fusarium, Alternaria, Claviceps and Chaetomium. The first pure mycotoxins isolated were ergot alkaloids (from 1875 on) and penicillic acid (in 1913). Others were isolated in the 1930's and 1940's, often without being toxicologically tested until later, when their usefulness as antibiotics was investigated. However, it was the discovery of the aflatoxins in 1962, following the outbreak of an associated mycotoxicosis in turkeys in England, that caused an acceleration in mycotoxin research. Not that this was the only natural mycotoxicosis that had been recognized up to that time. Various fusariotoxicoses of man and animals, ergotism, facial eczema in ruminants, and moldy corn toxicosis, to name a few, had been extensively investigated. Today, the subject of mycotoxins and mycotoxicoses is covered by a vast literature, which has been reviewed in books and journal articles, most recently by Rodricks et al. (1977), Wyllie and Morehouse (1977, 1978), Bullerman (1979), Moreau (1979), Stoloff (1979, 1983a), Cole and Cox (1981), and Ciegler and Vesonder (1983). Mycotoxins know no national boundaries, although development of mycotoxigenic fungi on agricultural commodities is influenced by climatic conditions. Mycotoxins of current importance in Canada include the trichothecenes and zearalenone (produced by Fusarium spp.), ochratoxin, ergot alkaloids, Chaetomium and Trichoderma metabolites thought to be associated with ovine ill thrift in Nova Scotia (Section 5.1.3), and, as "imported" mycotoxins, the aflatoxins. Selected toxins are discussed briefly below.

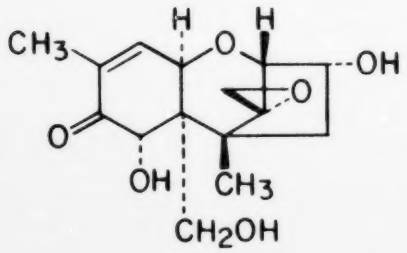
1.2 TRICHOTHECENES (H.L. Trenholm, R.M.G. Hamilton, D.W. Friend and E.R. Farnworth)

The term "trichothecenes" refers to a group of over 50 chemically related sesquiterpenoids (Figure 1-1), produced as secondary metabolites (12,13-epoxytrichothecene mycotoxins) by a broad range of fungal genera, e.g. Cephalosporium, Fusarium, Myrothecium, Stachybotrys and Trichoderma (Ueno 1977, 1980; Ribelin 1978; Pathre and Mirocha 1979; Booth 1982). The non-macrocyclics, e.g. diacetoxyscirpenol (DAS), fusarenon-X, HT-2 toxin, nivalenol, neosolaniol, T-2 toxin and deoxynivalenol (DON, vomitoxin), and the macrocyclics, e.g. verrucarin A and roridin A, are members of the trichothecene family (Tamm 1977). Clinical signs depend on a number of variables, including the toxins present, their levels, and the time period of ingestion, as well as on the species and nutritional-health status of the domestic and laboratory animals involved. Frequently associated with trichothecenes are nausea,

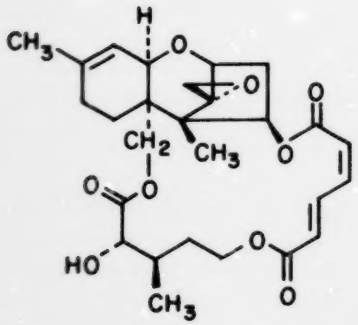


$R_1 = R_2 = CH_3COO$, $R_3 = (CH_3)_2CHCH_2COO$
$R_1 = OH$, $R_2 = CH_3COO$, $R_3 = (CH_3)_2CHCH_2COO$
$R_1 = R_2 = CH_3COO$, $R_3 = H$

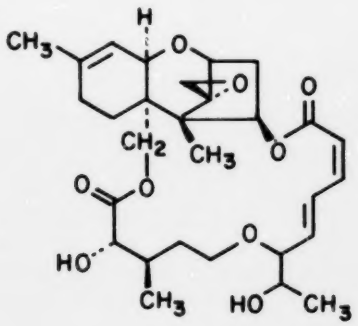
T-2 TOXIN, HT-2 TOXIN, DIACETOXYSCIRPENOL (DAS)



DEOXYNIVALENOL (DON, VOMITOXIN)



VERRUCARIN A



RORIDIN A

Figure 1-1. Chemical structures of some 12,13-epoxytrichothecene mycotoxins (T-2 toxin, HT-2 toxin, diacetoxyscirpenol, deoxynivalenol, verrucarin A and roridin A).

emesis, decreased feed consumption, feed refusal, skin irritation, diarrhea, hematological changes (e.g. leukopenia), hemorrhaging and reproductive problems (Ohtsubo and Saito 1977; Ciegler 1978). Trichothecenes are also potent inhibitors of protein and DNA synthesis (McLaughlin et al. 1977) and are known to cause immunosuppression (see also Sections 5.1.2-3 and 6.3.2.1).

Human and animal health regulatory officials are concerned about trichothecenes. Since the fungi involved are widely dispersed, the toxins have been found in several cereals, grains and feeds. There have been reports of illness in humans and in farm animals associated with ingestion of or exposure to trichothecenes. Canadian investigators have suspected trichothecene mycotoxicoses in livestock (Section 2.1).

1.3 ZEARALENONE (H.L. Trenholm, E.R. Farnworth and D.W. Friend)

Zearalenone ((E,S)-2,4-dihydroxy-6-(6'-oxo-10'-hydroxy-1-undecenyl)-benzoic acid-u-lactone (Figure 1-2)) is a mycotoxin produced by Fusarium molds (Neish et al. 1982) on plants such as corn and wheat, in the field and during storage. Key factors that seem to influence fungal invasion and subsequent zearalenone production on crops are insect damage, cool and damp weather conditions, mechanical damage during harvesting, and improper storage conditions (Mirocha et al. 1977; Naik et al. 1978; Sutton et al. 1980a, b).

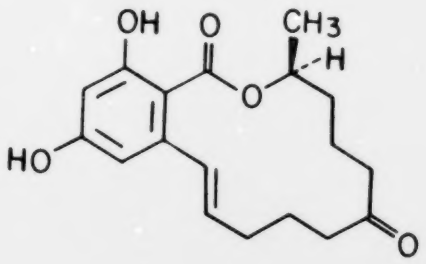


Figure 1-2. Chemical structure of zearalenone.

Zearalenone is an estrogenic compound that causes reproductive problems in farm animals, especially swine. Symptoms may include vaginal swelling (vulvovaginitis) and, in severe cases, vaginal and rectal prolapses, especially in immature gilts (swine). In young male swine, testicular atrophy and enlargement of the mammary glands may occur. Zearalenone ingestion may also cause prolonged estrus (heat) and reduced sex drive (libido), infertility, fetal mummification, stillbirths, reduced litter size and "splaylegs" (incoordination of the hind limbs) (Kurtz and Mirocha 1978). There is little evidence that zearalenone-contaminated diets affect feed intake or weight gains, or are a direct cause of death.

Zearalenone contamination is of particular importance to the agriculture and food industries for two reasons. First, zearalenone ingestion by livestock may cause losses in terms of reproductive performance and health (Nelson et al. 1973; Harwig and Munro 1975; Ruhr et al. 1978; Shreeve et al. 1978; Pier et al. 1980). In surveys carried out during 1973-74, it was estimated that hog production alone in Ontario, Canada may have lost more than one million dollars as a result of zearalenone contamination (Hunter 1983). Second, Zearalenone residues and its metabolites in plant and animal food products may be of concern in terms of human food safety. Zearalenone has been detected in grain dust in the United States, but routine exposure of grain handlers to it would be considerably lower than the amounts that produce minimal effects in non-human primates (Palmgren et l. 1983).

1.4 AFLATOXINS (P.M. Scott)

The four major aflatoxins (B_1, B_2, G_1, G_2) are structurally related metabolites of certain strains of Aspergillus flavus and A. parasiticus (Figure 1-3). Aflatoxins B_1, B_2 and G_1 have been shown to be carcinogenic in test animals. Foodstuffs most frequently found to contain aflatoxins are peanuts, cottonseed, corn, and tree nuts such as pistachios and Brazil nuts, produced in warmer areas of the world than Canada, with much of the contamination occurring before or during harvest (Stoloff 1979). Thus, the concern of regulatory authorities in Canada with respect to aflatoxins centers on imported foods. There is little evidence of aflatoxin occurrence in Canadian animal feedstuffs (Prior 1976).

Aflatoxin B_1, usually the main aflatoxin formed in foodstuffs, induced malignant tumors in different animal species, including rats, mice, monkeys, marmosets, ducks and rainbow trout (IARC 1976). The liver is the target organ, but other types of tumors have also been observed. A dose as low as 1 $\mu g.kg^{-1}$ of aflatoxin B_1 in the diet induced liver tumors in the most susceptible species of rat. Aflatoxins B_2 and G_1 are also known to induce tumors in rats, but there is no evidence that aflatoxin G_2 is a carcinogen. Aflatoxin M_1 (Figure 1-3), a metabolite of aflatoxin B_1 found in milk and milk products, is considered to be a less potent carcinogen than aflaxtoxin B_1. Imported cheeses are the only known source of aflatoxin M_1 in the Canadian diet. Levels of less than 1 $\mu g.kg^{-1}$ aflatoxin M_1 have been found in these cheeses.

A question frequently asked is whether aflatoxin is carcinogenic in man. In fact, high consumption of aflatoxins has been found in areas of the world with a high incidence of liver cancer such as Mozambique, parts of Thailand, Kenya, Swaziland, and the Philippines. An association between ingestion of increasing levels of aflatoxin and the rising risk of developing primary liver cancer has been demonstrated in epidemiological studies in those countries (Carlborg 1979; Bulatao-Jayme et al. 1981, 1982). However, a recent study within the population of the United States showed no significant increased risk of

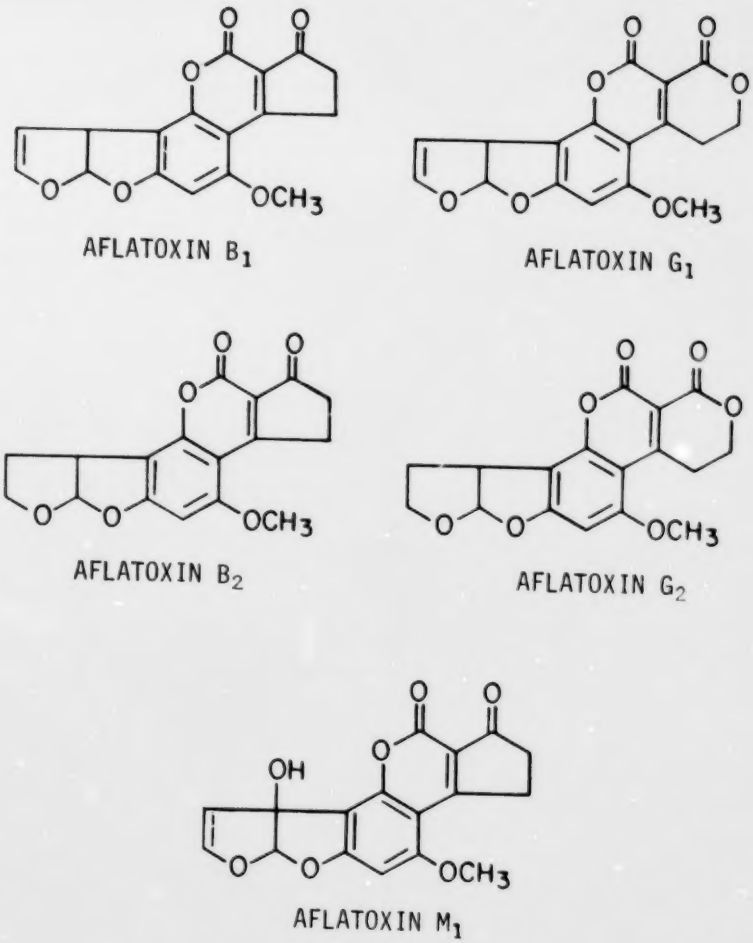


Figure 1-3. Chemical structures of aflatoxin B_1, aflatoxin G_1, aflatoxin B_2, aflatoxin G_2 and aflatoxin M_1.

primary liver cancer in the Southeast associated with the consumption of aflatoxin-contaminated corn products (Stoloff 1983b). Tentative associations of aflatoxin with other human diseases - hepatitis in India (Bhat and Krishnamachari 1977), kwashiorkor in children in Sudan (Hendrickse et al. 1982), Reye's syndrome (Nelson et al. 1980), lung cancer (Dvorackova et al. 1981), and other illnesses (Campbell and Stoloff 1974) - have been reported; the associations are not necessarily causative. There is some evidence that inhalation of aflatoxin-contaminated dust from a Dutch peanut and linseed processing plant was associated with cancer in the workers (Baxter et al. 1981; Burg et al. 1981).

Detoxification of foods contaminated with aflatoxin is not possible without affecting consumer acceptability. However, oilseeds such as cottonseed and corn have been treated with ammonia to effectively destroy aflatoxin, if present, in order to salvage those commodities for use as animal feed (Norred 1982; Price et al. 1982). Aflatoxins are only partially destroyed during normal food processing such as the roasting of peanuts. No practical means have been discovered for prevention of preharvest aflatoxin contamination in susceptible commodities, a responsibility in the hands of the producing countries, but the sorting of discolored nuts should be part of good manufacturing practice and is required in Canada.

1.5 STERIGMATOCYSTIN (P.M. Scott, D. Brewer and A. Taylor)

Sterigmatocystin (Figure 1-4), structurally related to the aflatoxins and a number of other fungal metabolites (Rodricks 1969), was first isolated from Aspergillus versicolor in 1954. This metabolite and its O-methyl derivative are intermediates in the biosynthesis of aflatoxins (Hsieh et al. 1973). Sterigmatocystin has been produced by a number of other fungal genera, including Chaetomium spp. (C. thielarioideum and C. udagawae, Udagawa et al. 1979a,b; Sekita et al. 1981) and Monocillium (Ayer et al. 1981). No significant natural occurrence of sterigmatocystin has been found (Stoloff 1983a), except in cheeses infested with A. versicolor (Northolt et al. 1980). In Canada, sterigmatocystin was detected in one sample of moldy wheat, and recently in a commercial mixed feed associated with mycotoxicosis in chickens

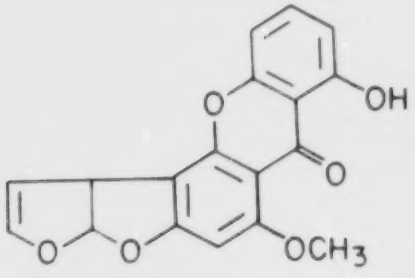


Figure 1-4. Chemical structure of sterigmatocystin.

(Abramson et al. 1983a) and in barley stored at 16 and 20% MC (Abramson et al. 1983b). Although acute oral toxicity of this mycotoxin is not high, it is hepatocarcinogenic in the rat, with a potency about one-tenth that of aflatoxin B_1.

1.6 ERGOT ALKALOIDS (J.C. Young)

Ergotism was undoubtedly the first mycotoxicosis known to man; records of its existence date back to antiquity (Bové 1970). Innumerable epidemics occurred throughout Europe, affecting up to 40 000 people at a time. Although the cause was first attributed to the sclerotia or "ergot bodies" of Claviceps spp. in 1673, epidemics continued into the early decades of this century. During the Middle Ages, those affected often turned to the monasteries for help, which included non-contaminated food. Some of the signs of witchcraft have also been attributed to ergot (e.g. Salem, Massachusetts in the late 1600's (Matossian 1982)).

Ergot has been termed a treasure chest of chemicals. Over 100 compounds in some 10 different groups are now known. The most physiologically active components are the ergot alkaloids (Figure 1-5), classified as derivatives of lysergic acid (1a) and its isomer (1b) or as clavine alkaloids based on the ergoline system 2.

When large amounts of ergot are consumed, convulsive symptoms including itching, numbness, severe muscle cramps and spasms, extreme pain and occasionally death are observed. When small amounts are consumed over an extended period, gangrenous symptoms include cramps, swelling, inflamation, alternating burning and freezing sensations ("St. Anthony's Fire"), and numbness; eventually the hands and feet may turn black, shrink and fall off.

A number of these alkaloids (Figure 1-5) have found therapeutic use in humans by virtue of their constricting effects on some muscle fibres and blood vessels and other effects on the central nervous system. For example, ergometrine (3) is occasionally used to stimulate labor, ergotamine (4) is used in the treatment of migraine headaches and to reduce postpartum bleeding, and the derivative 2-bromo-α-ergocryptine (5) is used in treating Parkinson's disease. Although symptoms of ergotism in humans due to natural causes are extremely rare today, there are occasional reports arising from drug overdoses (Harrison 1978). The artificial hallucinogen LSD (6) is a lysergic acid derivative.

Surveys of Canadian ergot (Young 1981a, 1981b; Young and Chen 1982) from a variety of grains and locations demonstrated the presence of at least a dozen alkaloids of the lysergic acid type. Ergocristine (7) and ergotamine (4) were the major components. Further details are discussed in Section 3.1.3 below.

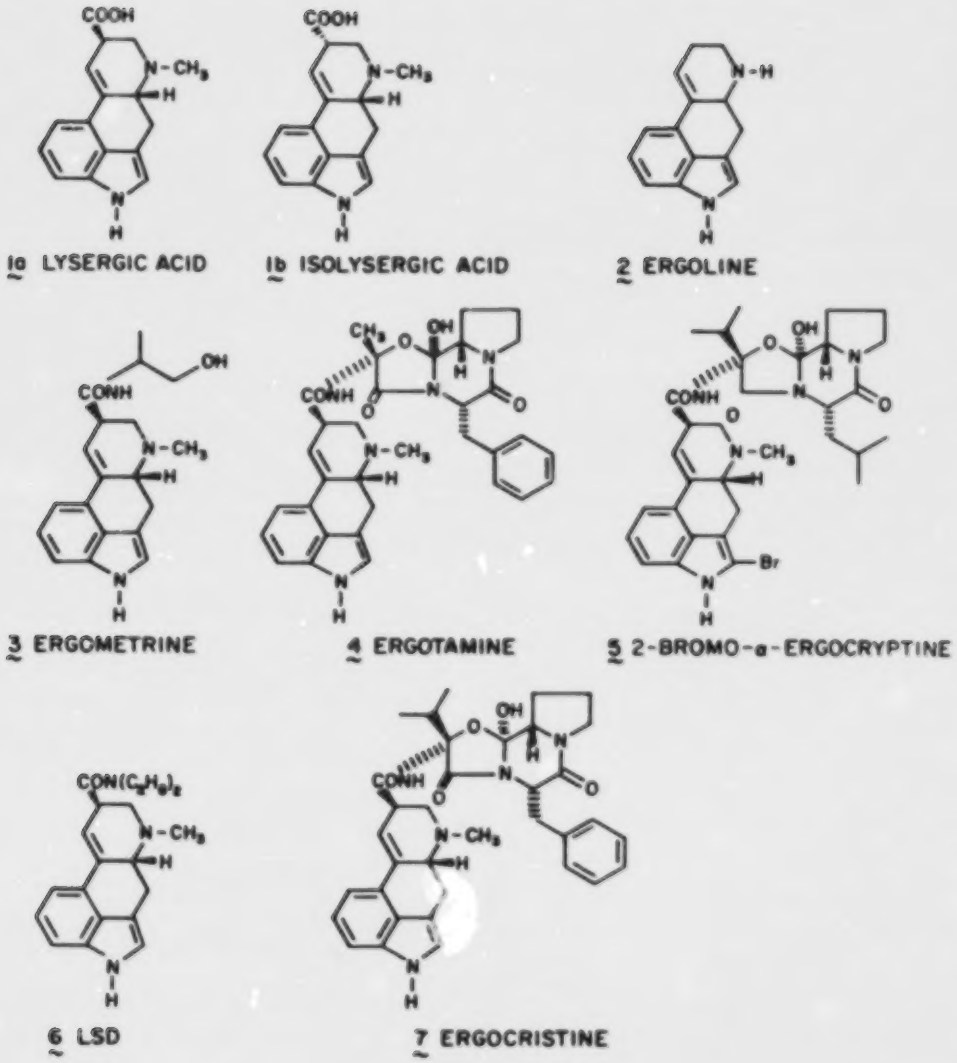


Figure 1-5. Chemical structures of ergot alkaloids and related compounds.

Although ergot sclerotia were found in samples of whole wheat for sale in some specialty food stores and alkaloids were discovered in some flours and baked products (Scott and Lawrence 1980, 1982), the greatest concern arising from ergot alkaloids relates to their occurrences in cereals destined for animal feeds.

Reviews on ergot include a comprehensive history (Bové 1970), and studies of its chemistry (van Rensburg and Altenkirk, 1974; Gröger 1978; Vining and Taber 1979; Floss and Anderson 1980; Rehacek 1980), occurrence in grains (Lorenz 1979; Seaman 1980), effects on animals (Wyllie and Morehouse 1977, 1978; Young 1979) and pharmacology (Berde and Schild 1978).

1.7 TENUAZONIC ACID (P.M. Scott)

The *Alternaria* species occur commonly on a wide variety of common food crops. There is increasing evidence of the occurrence of *Alternaria* mycotoxins under natural conditions. Tenuazonic acid (Figure 1-6) has the greatest acute toxicity of the various known *Alternaria* metabolites (Harvan and Pero 1976). Young chickens fed for 3 weeks with feed containing 10 $\mu g.g^{-1}$ or intubated daily with a 1.25 $mg.kg^{-1}$ dose had marked lesions in various tissues, including hemorrhages in the liver and kidneys and on the surface of the heart (Giambrone *et al.* 1978). Tenuazonic acid was the main *Alternaria* toxin produced in naturally infected tomatoes, but was found in lesser amounts than alternariol and its monomethyl ether in naturally infected apples (Stinson *et al.* 1981). Other fruits can also serve as good substrates for tenuazonic acid production (Stinson *et al.* 1980, 1981). Its presence (together with that of an isomer) in commercial tomato pastes on the Canadian market at concentrations of 0.01-0.1 $\mu g.g^{-1}$ (Scott and Kanhere 1980) indicates that further work is needed on the toxicology and occurrence in foods of tenuazonic acid. Comparison of the toxicity of isomeric tenuazonic acids is limited to an observation that sodium tenuazonate, which has an oral LD_{50} in rats and mice of 81-186 $mg.kg^{-1}$, was no longer lethal to mice after standing 4-5 months at room temperature; isomerization would probably have occurred after this time, as it does for the free acid. The total picture of contamination of foodstuffs by *Alternaria* toxins

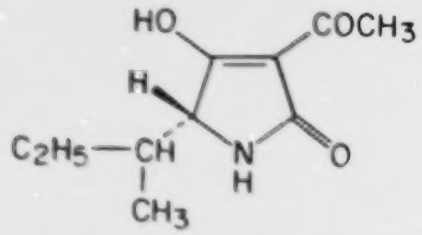


Figure 1-6. Chemical structure of tenuazonic acid.

should be taken into consideration, as crude fungal extracts as well as some specific toxins (not tenuazonic acid) are mutagenic in the Ames test (Scott and Stoltz 1980). In view of the demonstrated association of mutagenic activity with carcinogenicity, the possibility that Alternaria cultures are carcinogenic warrants further investigation.

1.8 OCHRATOXINS (P.M. Scott)

Ochratoxin A (Figure 1-7) and related metabolites were first isolated from a toxigenic strain of Aspergillus ochraceus in South Africa. Later evidence indicated that species of Penicillium, especially P. viridicatum and P. purpurescens, are the major molds responsible for contamination of grains and other foodstuffs by ochratoxin A, chiefly in countries with a temperate or continental climate, including Canada. Since the initial findings of ochratoxin A in heated grains from Saskatchewan farms at concentrations up to 27 $\mu g.g^{-1}$ (in some samples accompanied by citrinin), there have been several major surveys of Canadian grains and suspect feedstuffs for this mycotoxin (see Section 2.2.3). These showed a low incidence. The presence of ochratoxin A in Ontario white beans has also been demonstrated. In other countries, the presence of ochratoxin A in feedstuffs and foodstuffs was associated with a number of outbreaks: mycotoxin porcine nephropathy was associated with ochratoxin in Danish barley, endemic human nephropathy was related to grains in Yugoslavia and mycotoxic avian nephropathy was associated with ochratoxin-contaminated feeds in Denmark and the United States. Ochratoxin A is thus of major importance to agri-food systems. It is sometimes present in the tissues of swine and poultry consuming contaminated feed. In Denmark, a pig carcass is condemned if the concentration of ochratoxin A in the kidney exceeds 25 $\mu g.g^{-1}$ (Schuller et al. 1982), and in Sweden, the observation of macroscopically abnormal kidneys at slaughter is recorded and ochratoxin analysis is subsequently carried out. In Canada, there are no regulations specifically pertaining to ochratoxin A and there are none for grain foodstuffs elsewhere.

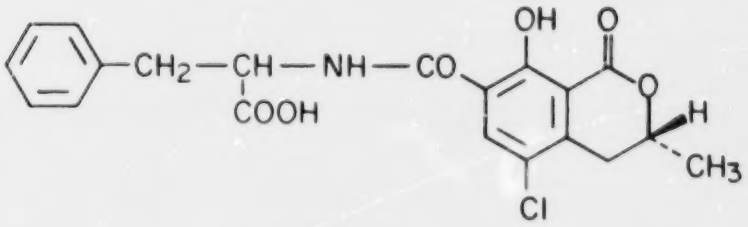


Figure 1-7. Chemical structure of ochratoxin A.

As indicated above, ochratoxin A affects primarily the kidney. It is embryotoxic and teratogenic in rodents and neoplastic changes have been reported in the liver and kidneys of mice given ochratoxin A in the diet. Most recent research on ochratoxin A has concentrated on its biological effects and these have been comprehensively reviewed, together with all other known aspects of the ochratoxins, by Harwig *et al.* (1983).

1.9 CITRININ (P.M. Scott)

Citrinin (Figure 1-8) was first obtained in 1931 from *P. citrinum* and has since been shown to be a metabolite of several other *Penicillium* species, certain *Aspergillus* species, and, curiously, an Australian herb, *Crotalaria crispata*.

Like ochratoxin A, citrinin causes kidney damage in experimental animals, but its toxicity is lower. Citrinin may play a minor role in porcine nephropathy in Denmark. Co-occurrence with ochratoxin A has been reported in some samples of barley or mixed barley-oats obtained from districts in Denmark with a high incidence of this disease, in heated grains from Saskatchewan, and in *Penicillium viridicatum* isolated from the latter grains (Scott 1978a). In addition to aflatoxins, citrinin was found in Indian groundnuts, in corn imported from Thailand and Burma to Japan, and in corn flour made from the Thailand corn (Scott 1978a, Nakazato *et al.* 1982); the groundnuts and corn were contaminated with *P. citrinum* and *A. flavus*. One recent survey for citrinin in Canadian grains has been carried out, with negative results (Abramson *et al.* 1983a).

1.10 PATULIN (P.M. Scott)

The antibiotic now generally known as patulin (Figure 1-9) was first isolated under the name claviformin in 1942 from *Penicillium claviforme*. There are several other synonyms reflecting multiple discovery of the compound as a common metabolite of *Aspergillus* and *Penicillium* species (Scott 1978b). It is also produced by *Byssochlamys* species. Patulin was classed as a mycotoxin because of its acute toxicity in rodents, and interest in it as a potential food contaminant arose because of observations of carcinogenicity on subcutaneous injection in rats and its production by *P. expansum*, a common storage rot of apples. Frequent occurrence of patulin in apple juice has been demonstrated by surveys, including one in Canada (Hanchay 1978). However, lack of tumorigenic activity of patulin administered orally to rats (Becci *et al.* 1981) has resulted in a lack of regulatory interest in North America, although certain countries - Norway, Sweden and Switzerland - have action levels of 50 $\mu g.L^{-1}$ of juice.

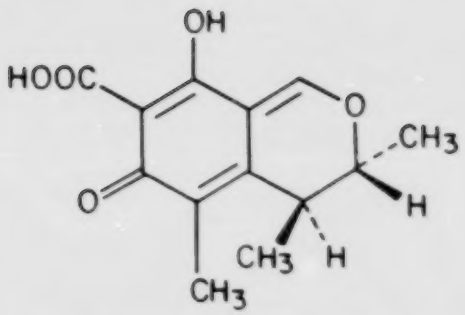


Figure 1-8. Chemical structure of citrinin.

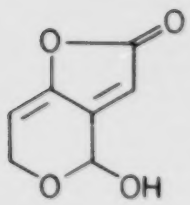


Figure 1-9. Chemical structure of patulin.

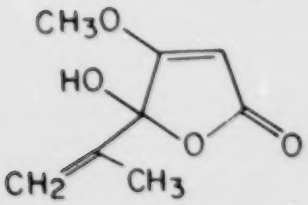


Figure 1-10. Chemical structure of penicillic acid.

1.11 PENICILLIC ACID (P.M. Scott)

Like patulin, penicillic acid (Figure 1-10) is an α,β-unsaturated lactone, a common metabolite of several Aspergillus and Penicillium species, and carcinogenic in rats by subcutaneous injection (Scott 1978b). However, survey effort has been very limited, although occurrence of this mycotoxin has been reported in corn, beans and tobacco in the United States. An analytical method remains to be validated.

1.12 CHAETOMIUM METABOLITES (D. Brewer and A. Taylor)

1.12.1 CHETOMIN

Chetomin (Figure 1-11) (Waksman and Bugie 1944; McInnes et al. 1976) has been isolated from cultures of Chaetomium cochliodes (originally cultured from soils collected in Nova Scotia), C. globosum (Brewer et al. 1972a), and C. subglobosum (Sekita et al. 1981). It is probably produced by C. funicola and C. umbonatum (Brewer and Taylor 1978). Corn invaded by C. globosum was lethal to rats (Christensen et al. 1966) and it was established that the toxic agent was chetomin (Brewer et al. 1972b). Chetomin has been shown to be toxic to rats (LD_{50} 50 $mg.kg^{-1}$, oral), turkey poults (LD_{50} 30 $mg.kg^{-1}$, oral), and ewe lambs (LD_{50} 5 $mg.kg^{-1}$, intraperitoneal) and to have an inhibitory effect at 0.02 $\mu g.ml^{-1}$ on the growth of cultures of epithelial cells (Trown 1968; Brewer et al. 1972b). Two related toxic metabolites have been isolated: dithiochetomin (Brewer et al. 1972b) and dethio-tetra-(methylthio)chetomin (Kikuchi et al. 1982).

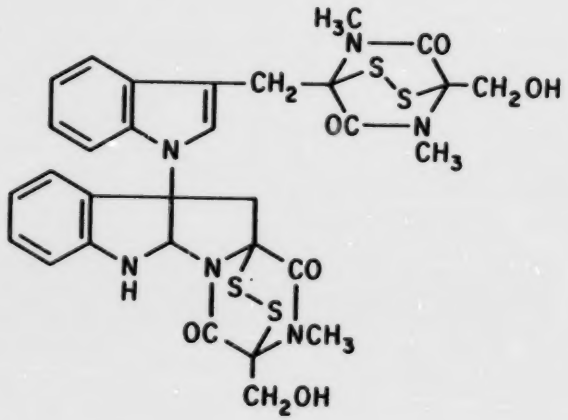


Figure 1-11. Chemical structure of chetomin.

1.12.2 CHAETOCIN

Chaetocin (Figure 1-12), has been isolated from Chaetomium minutum (Hauser et al. 1970), C. thielavioideum and Farrowia sp. (Udagawa et al. 1979b). It has also been shown to be produced by Achaetomiella virescens (Sekita et al. 1981). The minimum concentration of chaetocin to produce 50% inhibition of growth of HeLa cells was 0.05 $\mu g.mL^{-1}$ (Udagawa et al. 1979b).

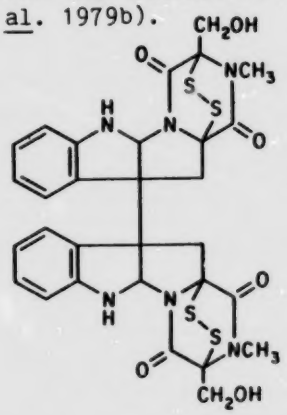


Figure 1-12. Chemical structure of chaetocin.

1.12.3 CHAETOGLOBOSINS

The chaetoglobosins A (Figure 1-13), B (Sekita et al. 1973), C, D, E, F (Sekita et al. 1976), G and J (Sekita et al. 1977), are cytochalasins derived from tryptophan and were originally isolated from Chaetomium globosum. They are now known to be produced (in various combinations) by isolates of C. cochliodes, C. globosum, C. mollipilium, C. rectum, and C. subaffine (Brewer and Taylor 1978; Udagawa et al. 1979b). Umeda et al. (1975) reported the cytotoxicity of chaetoglobosins and Ohtsubo et al. (1978) observed the acute toxicity of chaetoglobosin A to rats injected intraperitoneally (LD_{50} 2 $mg.kg.b.w.^{-1}$) and mice injected subcutaneously (LD_{50} 6.5 $mg.kg.b.w^{-1}$. male and 17.8 $mg.kg.b.w.^{-1}$ female).

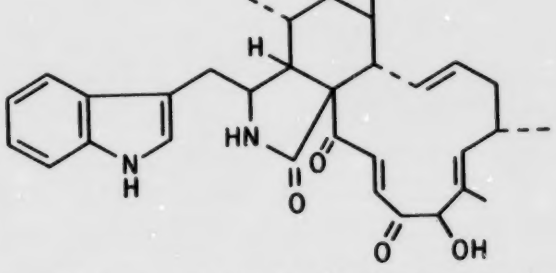


Figure 1-13. Chemical structure of chaetoglobosin A.

Chaetoglobosin K has been isolated from cultures of Diplodia macrospora, the causal organism of an ear rot and stalk rot of corn (Cutler et al. 1980). This compound is toxic to day-old chicks (LD_{50} = 40 $mg.kg^{-1}$, oral).

1.12.4 OOSPOREIN

Oosporein (Figure 1-14) was originally isolated from cultures of Oospora colorans (Kögl and van Wessem 1944). It was produced also by Chaetomium aureum (Lloyd et al. 1955), C. trilaterale (Cole et al. 1974), and Beauveria bassiana (Vining et al. 1962). The toxicity of oosporein to fungi (Brewer et al. 1977) and cockerels, oral LD_{50} 6.12 $mg.kg^{-1}$ (Cole et al. 1974), has been demonstrated.

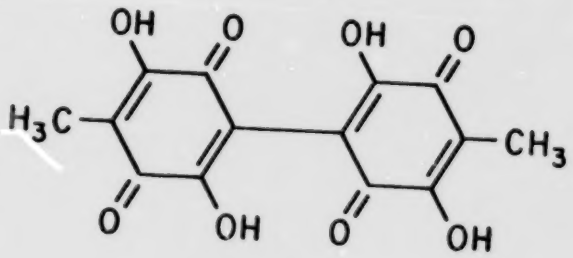


Figure 1-14. Chemical structure of oosporein.

1.12.5 COCHLIODINOL

Cochliodinol (Figure 1-15) (Jerram et al. 1975; Hanson 1977) is produced by Chaetomium cochliodes, C. globosum (Brewer et al. 1968), and C. elatum (Sekita et al. 1981). It inhibits the growth of fungi, e.g. Fusarium oxysporum, at ≈ 10 $\mu g.ml^{-1}$ and bacteria, e.g. Pseudomonas aeruginosa, at ≈ 30 $\mu g.ml^{-1}$, but it required 10 $ug.ml^{-1}$ to inhibit the growth of epithelial cells and ≳ 2 $g.kg^{-1}$ to affect the growth of young rats (Brewer et al. 1970).

Two related metabolites, isocochliodinol and neocochliodinol, have been isolated from Chaetomium murorum and C. amygdalisporum, respectively (Sekita et al. 1981).

1.12.6 CHAETOCHROMIN

Chaetochromin (Figure 1-16) was originally isolated from Chaetomium thielavioideum (Sekita et al. 1980). It has also been isolated from C. caprinum, C. gracile, and C. tetrasporum (Sekita et al. 1981). It is cytotoxic to HeLa cells (at 3 $\mu g.ml^{-1}$) and acutely toxic to mice (at 10 $mg.kg^{-1}$) (Sekita et al. 1980).

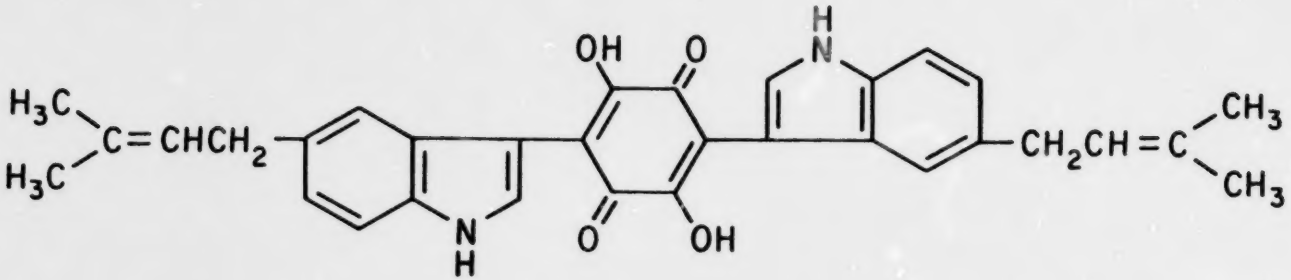


Figure 1-15. Chemical structure of cochliodinol.

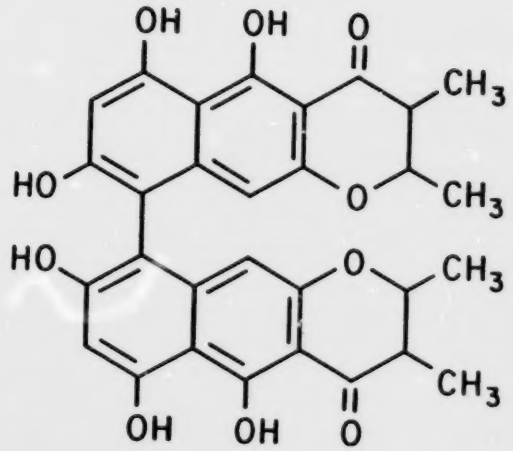
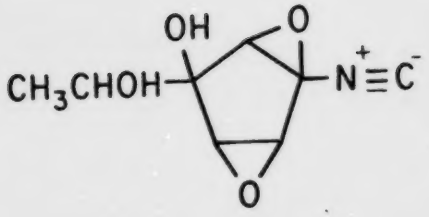
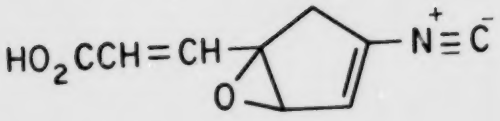


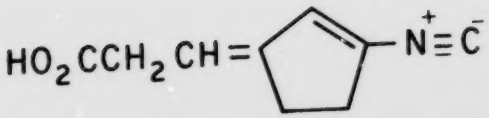
Figure 1-16. Chemical structure of chaetochromin.



TRICHOVIRIDIN



DERMADIN



ISOCYANIDE III

Figure 1-17. Chemical structure of trichoviridin, dermadin and isocyanide III.

1.13 ISOCYANIDES (D. Brewer and A. Taylor)

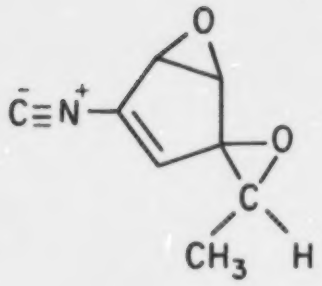
The compounds noted here, which are characterized by an isocyanide functionality, are a large group of toxic metabolites produced by many species of Trichoderma. Trichoviridin (Figure 1-17) was isolated from cultures of Trichoderma sp. (Nobuhara et al. 1976) and dermadin (Figure 1-17) and isocyanide III (Figure 1-17), together with trichoviridin, were isolated from T. hamatum cultures (Brewer et al. 1982). Trichoviridin and dermadin are toxic to mice when given intraperitoneally; the LD_{50} values are 100 $mg.kg^{-1}$ and 240 $mg.kg^{-1}$, respectively (Fujiwara et al. 1982). Trichoviridin is produced by cultures of T. koningii and dermadin by T. koningii, T. harzianum, and T. polysporum (Fujiwara et al. 1982, Okuda et al. 1982). A number of other isocyanides produced by Trichoderma spp. are commonly found in soils in Nova Scotia. These include isonitrin A (Figure 1-1; LD_{50} of 160 $mg.kg^{-1}$, intraperitoneal in mice), isonitrin B (Figure 1-18; LD_{50} of 300 $mg.kg^{-1}$, intraperitoneal in mice), isonitrin D, and the homothallins (Anderson et al. 1979, Fujiwara et al. 1982).

1.14 OTHER MYCOTOXINS IN HUMAN FOODS AND ANIMAL FEEDS (P.M. Scott)

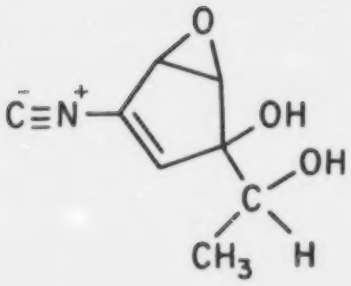
Many other mycotoxins have been discovered and described in reviews (see Section 1.1). Few have been detected in foodstuffs. However, mold-ripened cheeses have recently been investigated intensively and certain alkaloids (roquefortine, isofumigaclavine A) and mycophenolic acid, metabolites of Penicillium roqueforti, have been detected in blue cheese, while cyclopiazonic acid has been found in the crusts of Camembert-type cheeses (Scott 1981a). No acute health hazard can be identified from these occurrences. Preliminary studies with rats indicated that PR toxin, one of the most acutely toxic metabolites of P. roqueforti, is tumorigenic (Polonelli et al. 1982), but fortunately, PR toxin is unstable in blue cheese. Cyclopiazonic acid has also been detected in U.S. peanuts and corn (Gallagher et al. 1978; Lansden and Davidson 1983). Secalonic acid D has been identified as a natural contaminant of grain dust obtained from grain storage elevators in the United States (Ehrlich et al. 1982). Both cyclopiazonic acid and secalonic acid D are produced by Penicillium species frequently found in grains - P. cyclopium (see Section 4.2) and P. oxalicum are the principal fungal contaminants of midwestern U.S. corn that could occur in Canadian grains as well.

1.15 BIOSYNTHETIC STUDIES IN CANADIAN MYCOTOXIN RESEARCH (J.D. Miller)

The study of secondary metabolism in fungi is a very complex matter. The number of genes involved in the biosynthesis of, for example, aflatoxin has been estimated to be upwards of 150. Most secondary metabolites are produced during the equivalent of stationary phase (idiophase), unbalanced growth induced by one or more nutrient limitations occurring together or in a specific sequence. Other



ISONITRIN A



ISONITRIN B

Figure 1-18. Chemical structure of isonitrin A and isonitrin B.

parameters (pH, O_2, CO_2, temperature, etc.) must be in a certain relationship after the nutrient limitations have occurred to allow the biosynthesis of a given metabolite (Bu'Lock 1975). It is believed that an accumulation of a toxic secondary metabolite in nature requires that a specific set of nutrient limitations and physical conditions occur in the microclimate around the hyphae (ca. 4 µm in diameter) penetrating the substrate.

Secondary metabolites of fungi are produced on metabolic grids. For many compounds, several pathways exist in equilibria, one being most important depending on the genetics of the isolate and environmental conditions, including available nutrients. Although it is not, strictly-speaking, a secondary metabolite, the C_{28} sterol ergosterol has 22 known pathways, but in most fungi, three are dominant (Weete 1980). The known metabolic grid of patulin described by Sekiguchi (1983) also illustrates this multiple pathway phenomenon. A consequence of this problem (i.e. multiple pathways) is that biosynthetic studies must first attempt to identify many of the minor intermediates that occur in the fermentation of the major compound. Natural occurrences of mycotoxins are mixtures of fungal metabolites with properties different from the dominant, known toxin. Methods that allow the accumulation of minor metabolites for toxicity studies have considerable value in improving the understanding of the toxicity of mycotoxin-contaminated foods and feed.

A requirement for most types of biosynthetic studies is a liquid fermentation method for the production of the metabolite of interest, preferably in a stirred tank fermentor. Detailed biosynthetic studies, although perhaps superficially esoteric, have a number of scientific and practical benefits. Procedures used to produce a metabolite on a small scale can usually be scaled up to provide quantities of the metabolite for large scale toxicological and chemical evaluation. In some cases, e.g. DON, the compounds are commercially unavailable in quantity. For other mycotoxins, costs are very high. It has been estimated that several hundred thousand dollars have been spent by Canadian scientists in recent years on importing mycotoxins for research in government and university laboratories.

When DON was identified in the 1980 white-winter-wheat crop in eastern Canada, fermentation methods were developed to undertake biosynthetic studies and produce DON and related compounds in sufficient quantity for animal toxicology studies. At the same time, a number of new compounds (with unknown toxicity) were isolated and described, including a new major precursor of DON (Miller *et al.* 1983a; Greenhalgh *et al.* 1984). Furthermore, Canadian isolates of *Fusarium graminearum* were shown to be different in their biosynthetic patterns from Japanese isolates. This illustrates a problem that could result from extrapolating information on the toxigenic potential of *Fusarium* isolates from one locale to another. It has been shown that plant enzymes have important effects on the synthesis of DON by the invading *F. graminearum*. These include effecting structural modifications to the

toxin, degrading the toxin and perhaps preventing its biosynthesis (Miller et al. 1983b, 1984).

Metabolic studies require and provide a means for the biosynthesis of radiolabelled compounds. These are valuable for animal metabolic studies and, when produced in fermentors, are considerably cheaper than other possible means of adding labels. Lastly, a detailed understanding of the biosynthetic processes of mycotoxins raises the possibility of interfering with the processes as a means of controlling mycotoxins in vivo.

General reviews of secondary metabolism in fungi can be found in Demain (1972), Bu'Lock (1975, 1980), Martin and Demain (1980), Herbert (1981) and Malik (1982). Discussions of the biosynthesis of certain mycotoxins including ergot toxins, aflatoxins and ochratoxin can be found in Steyn (1980).

In Canada, unfortunately, very little work is done on the biosynthesis of mycotoxins. With the exception of sporadic reports on aflatoxin (Nakashima and Vederas 1982) and some other mycotoxins, only patulin and some Fusarium mycotoxins have been seriously studied in Canada with respect to their biosynthesis.

Patulin

Gaucher et al. (1981 inter alia) published several reports on patulin biosynthesis and have made an important contribution to the understanding of the pathways involved (cf. Sekiguchi 1983). A review of patulin biosynthesis can be found in Zamir (1980).

Fusarium Mycotoxins

As noted in 5.1.2, Fusarium mycotoxins are the most important to Canadian agriculture. The biosynthesis of zearalenone has been described (Vleggaar and Steyn 1980) and the compound is commercially available.

What is known about the biosynthesis of trichothecenes has been described by Tamm and Breitenstein (1980). A detailed discuss on of the biology of Fusarium toxin production appears in a report of the U.S. National Academy of Science (Anonymous 1983). There are many unresolved problems in the biosynthesis of specific trichothecenes and much research is required. Japanese and American researchers have made many contributions concerning the chemistry and toxicology of trichothecenes (cf. Ueno 1983) but essentially no effort has been put into biosynthetic studies. A major unresolved problem is the question of the variable toxicity of naturally contaminated grain. Recent reports have implied that DON-contaminated grain demonstrates more serious toxic effects than actually can be proven under controlled conditions (Côté et al. 1984; cf. section 5 this report). This strongly suggests that other compounds produced by Fusarium graminearum (or

associated trichothecene-producing Fusaria) are contributing to the toxicity. The last issue that requires consideration is the actual biosynthesis of trichothecenes in crop plants.

Canada has a major research program in the biosynthesis of the trichothecene DON and related compounds involving scientists from Agriculture Canada, NRCC and Institute Armand Frappier. The other Fusarium toxins potentially of concern to Canada, e.g. T-2 toxin, HT-2 toxin and F. moniliforme toxin, are not studied at all. The following is a brief summary of the progress to date.

In vitro: Fermentation methods have been developed to undertake biosynthetic studies and to produce DON and related compounds in quantity (Miller et al. 1983a; Greenhalgh et al. 1984). The effects of different nutrients on trichothecene production by different species of F. graminearum have been examined (Miller and Greenhalgh 1984). In addition, a number of new compounds (with unknown toxicity) have been described including a new major precursor of DON (Greenhalgh et al. 1984; Miller et al. 1983a). Canadian strains of F. graminearum were shown to be different in their biosynthetic patterns than Japanese isolates, illustrating the danger of extrapolating information on the toxigenic potential of Fusarium isolates tested in other locales. Studies are in progress using ^{13}C N.M.R. techniques (Blackwell et al. 1984) and ^{14}C pulse-labelling methods.

In vivo: Several studies have been done on the formation of DON etc. in experimental and natural infections of wheat and corn with F. graminearum. It has been shown that plant enzymes have important effects on the synthesis of DON by the invading F. graminearum. These include effecting structural modifications to the toxin, degrading the toxin and perhaps preventing its biosynthesis (Miller et al. 1983b, 1984). This research shows great promise for future developments. Preliminary experiments have been done in vitro and in vivo with the application of compounds to prevent toxin synthesis.

2.0 OCCURRENCE OF MYCOTOXINS IN CANADA

2.1 MYCOTOXINS IN FEEDS AND FEEDSTUFFS[a] (H.L. Trenholm, B.K. Thompson, J.F. Standish and W.L. Seaman)

Before embarking on a major research program on the nutritional and toxicological effects of mycotoxins on livestock and poultry, the Animal Research Centre of Agriculture Canada awarded a contract to Deloitte, Haskins and Sells Associates in 1978 to complete a national survey on mycotoxins. The objectives were to determine the extent and nature of mycotoxin occurrence in feeds and feedstuffs and to define animal health problems associated with the ingestion of mycotoxin-contaminated feeds. The survey included a review of data and published reports from federal and provincial laboratories, as well as from universities and private companies across Canada (Andrews et al. 1981).

In addition to the national survey there are several reports indicating the natural occurrence of mycotoxins in Canadian feeds and feed ingredients (Scott et al. 1972; Greenway and Puls 1976; Prior 1976, 1981; Puls and Greenway 1976; Trenholm et al. 1981b; Abramson et al. 1983a) and of molds capable of producing mycotoxins (see Sections 3.0 and 4.0). Although Trenholm et al. (1982) indicated that there has not been a well documented case of a mycotoxin being identified as the specific cause of livestock illness, most of the reported mycotoxin analyses, with the exception of surveys for DON, have been conducted on samples that have been associated with livestock or human illnesses. The findings of the national survey, as well as of other reports and more recent information, are included in this section.

The provincial government of British Columbia analyzed 200 samples for mycotoxins during the period of 1970-1977. Sixty-five of the samples, with 21 positives for T-2 toxin, were taken in the Peace River area where Fusarium contamination of barley was a problem (Greenway and Puls 1976). In October 1973, there were suspected mycotoxicoses involving domestic ducks, geese, horses and swine in the Peace River District of northern British Columbia and Alberta. All species showed upper alimentary distress with deaths occurring in geese. The clinical symptoms and analytical data (25 $\mu g.g^{-1}$ of T-2 toxin was identified by thin layer chromatography (TLC) in an associated sample of barley) indicated T-2 toxicosis (Puls and Greenway 1976). In another incident that year, ochratoxin was found in mink feed but never linked directly to the final diagnosis.

In samples analyzed at the Alberta government laboratory during the period 1973 to 1978, only trace levels of aflatoxin, sterigmatocystin, ochratoxin and zearalenone were detected in a very

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[a] A.R.C. Contribution No. 1274.

small number of the samples. In 1975 there was an outbreak of mycotoxicosis in swine in the Peace River area. Although T-2 toxin was not identified, feeding trials with hogs, chickens and geese brought about symptoms consistent with T-2 toxin ingestion (Andrews et al. 1981).

In 1971 mycotoxin testing facilities were established at the Animal Pathology Laboratory, Agriculture Canada in Saskatoon, Saskatchewan, to assist Western Canadian farmers who were concerned about the effects of mycotoxins on livestock production. Over the years, molds infected surplus stored grain, and as an alternative to selling the downgraded infected grain at reduced prices, farmers began raising cattle to utilize the surplus. During the period 1971-1975, 190 samples were submitted to the laboratory because of concern about moldy feedstuffs or suspected mycotoxicoses (Prior 1976). In the period 1975-1979, 499 samples were analyzed for mycotoxins. The results for these tests are summarized in Table 2-1. The mycotoxin levels identified suggested a greater probability for chronic or subclinical mycotoxicosis associated with the low incidence of mycotoxins in the suspected animal feedstuffs than for acute outbreaks of mycotoxicoses at elevated mycotoxin levels.

In a separate study, Scott et al. (1972) reported mycotoxins in grain and feed samples associated with lung problems in farmers and elevator operators, and sickness or death in farm animals. Ochratoxin A was detected at levels ranging from 0.03 to 27 $\mu g.g^{-1}$ in 18 out of 29 samples of heated grain from Saskatchewan farms. The grain was harvested in 1968 and stored under damp conditions which led to heating and spoilage. Thirteen of the above samples also contained citrinin at levels ranging from 0.07 to 80 $\mu g.g^{-1}$. No penicillic acid was reported, despite the fact that Penicillium molds capable of producing this compound were isolated from many of the samples. Only one sample indicated the presence of trace amounts of aflatoxin B_1. Sterigmatocystin was found in just one grain sample. In only one sample known to be associated with deaths of farm animals was an appreciable concentration of ochratoxin A determined (0.53 $\mu g.kg^{-1}$). The sample was taken from a moldy mixed feed suspected of causing the death of a cow and several calves; in addition, milk production in the herd was unusually low. In all, ochratoxin A was detected in 2 of 13 mixed feeds, plus 3 samples of dried white beans.

Abramson et al. (1983a) analyzed feed samples from 51 suspected cases of mycotoxicoses of farm animals in western Canada during the period January 1979 to December 1981. Ochratoxin A (140 $\mu g.kg^{-1}$) was detected in a pelleted commercial feed for 8800 broiler chicks experiencing increased mortality, poor weight gains and organ damage. T-2 toxin (2.5 $\mu g.g^{-1}$), diacetoxyscirpenol (DAS) (0.8 $\mu g.g^{-1}$) and ochratoxin A (380 $\mu g.kg^{-1}$) were found in a dairy ration; symptoms in 2 cows included diarrhea, depression and prostration. In one instance

Table 2-1. Detection of mycotoxins in feed samples at the Animal Pathology Laboratory, Agriculture Canada, Saskatoon.

Period	Number of samples tested	Type of sample tested	Type of test	Mycotoxin tested for	Number of samples contaminated	Level of mycotoxin ($\mu g.kg^{-1}$)	Reference
1971-1975	100	Alfalfa brome hay	chemical	Aflatoxin $_1$	1	50	Prior (1976)
	95[α]	Wheat	"	Ochratoxin A	6	30-6000	"
		Hay	"	"	1	30	"
1975-1979	499[β]	Hay	"	Afaltoxin B_1	1	60	Prior (1981)
		Rye	"	Ergot alkaloids	3	-[γ]	"
		Farm-mixed fsed	"	"	1	-	"
		Grain	"	Ochratoxin A	4	30-4000[δ]	"
		Forage	"	"	1		
		Grain	"	Zearalenone	1	500	
		Corn	"	"	2		
		Forage	Skin bio-assay	Tricothecenes	2	-	"
		Feed	"	"	3	-	"
		Grain	"	"	1	-	"

[α] Total wheat and hay samples.

[β] Total of all types of samples for the period 1975-1979.

[γ] Indicates level not known.

[δ] Range of level of Ochratoxin A in both grain and forage samples.

involving 1600 layers, decreased feed consumption, decreased egg production and liver damage were reported and sterigmatocystin (2.3 $\mu g.g^{-1}$) was found to be present. When 40 dairy cattle showed symptoms of decreased milk production (27%), retained placentae (50%) and feed refusal, the high moisture barley ration was found to contain 48 $\mu g.kg^{-1}$ ochratoxin A. In another case concerning beef cattle fed wheat, oats and barley, ochratoxin A was detected at 5.9 $\mu g.g^{-1}$, but no clinical signs were observed.

In western Canada, development of mycotoxins may occur in stored cereal grains when particular ecological conditions occur. The likelihood of mycotoxin development increases during the storage of certain types of cereals, particularly when the grains are binned at high moisture and temperature and when moist pockets develop in the grain through moisture migration (Mills 1982). The short growing season in certain areas of the Peace River Valley combined with frequent rains in September and October can create ideal conditions for mold infection and mycotoxin production. Moist, humid regions of southern Manitoba, especially in the Red River Valley, constitute another area where one would expect problems with mycotoxins.

Mycotoxins may be more prevalent in certain humid southern areas of eastern Canada. The Ontario Veterinary Services laboratory analyzed over 2000 feed samples suspected of mycotoxin contamination during the period 1972-1977. One or more mycotoxins were detected in 13.7% of the samples. The most commonly detected mycotoxin was zearalenone at an average level of 3.85 $\mu g.g^{-1}$ with a range of 0.01-141 $\mu g.g^{-1}$ (Funnell 1979; Sutton et al. 1980a, b). Corn is a principal ingredient of swine feed in the province of Ontario. During the 6-year period, from 9 to 29% of corn samples were positive for zearalenone (Sutton et al. 1980a). At the higher levels of zearalenone found in the feed samples, one would expect estrogenic effects, especially in swine. Most of the specimens (55 to 75% over the period studied) were feed samples from swine operations. In fact, the main mycotoxicosis reported was vulvovaginitis in young gilts (females), a symptom characteristic of zearalenone estrogenism. Funnell (1979) detected aflatoxin in 7 samples and ochratoxin in 3 samples.

In order to prevent reproductive problems in their farrowing herds, producers avoid feeding home-grown corn to their breeding sows and gilts. This disrupts the total integration of swine operations, since they have to purchase grain from unaffected areas, such as western Canada, where zearalenone contamination does not occur.

In 1980, there were reports of Fusarium in the Ontario white winter wheat and the Québec spring wheat crop. Fusarium head blight infections in 1980 reduced cereal crop yields in the Atlantic provinces (Martin and Johnston 1982).

The visual presence of Fusarium mold on the 1980 Ontario white winter wheat crop led to several fairly extensive surveys for DON in Canadian wheat. Scott et al. (1981) found DON in 44 of 45 samples of 1980 Ontario winter wheat in concentrations ranging from 0.01 to 4.3 $\mu g.g^{-1}$ and in all of 27 samples of 1980 Québec red spring wheat (85% ≥ 0.3 $\mu g.g^{-1}$); wheat from western Canada (Alberta, Saskatchewan and Manitoba) was not a problem based on an analysis of 25 samples. Trenholm et al. (1981b) reported mean DON levels of 0.38 $\mu g.g^{-1}$ in 9 samples of Ontario white winter wheat graded from No. 2 Canada Eastern to No. 5 Canada Eastern, 0.93 $\mu g.g^{-1}$ in 28 samples of Canada Feed Grade wheat and 0.91 $\mu g.g^{-1}$ in 4 samples of ungraded wheat. Levels as high as 8.5 $\mu g.g^{-1}$ were reported in these samples, obtained from individual producers' shipments to country and terminal elevators. That these samples may not have been representative of the entire Ontario crop is indicated by the fact that samples of boatloads destined for export markets showed levels of only 0.15 to 0.16 $\mu g.g^{-1}$ of vomitoxin. Other mycotoxins such as citrinin, ochratoxin, zearalenone, sterigmatocystin, aflatoxins B_1, B_2, G_1 and G_2, penicillic acid, rubratoxin, luteoskyrin and patulin were not found in the 41 individual producers' samples when the acidified acetonitrile extraction-thin layer chromatography screening method was used.

More rigorous surveys of Canadian wheat were conducted in 1981 and 1982 by Agriculture Canada, Health and Welfare Canada and the Québec Ministry of Agriculture, Fisheries and Food. Sampling intensity was roughly proportional to area production levels, so as to be indicative of the entire crop. DON levels averaged 0.18 $\mu g.g^{-1}$ in the 1981 Ontario white winter wheat crop and 0.70 $\mu g.g^{-1}$ in the 1982 crop. In Québec spring wheat, the average levels determined were 2.27 $\mu g.g^{-1}$ in 1981 and 0.28 $\mu g.g^{-1}$ in 1982. Actually, one sample representing a lot of 135 tonnes of Québec wheat was analyzed and found to contain 7.71 $\mu g.g^{-1}$ vomitoxin. If this sample is included, the mean for the Québec samples in 1982 would be 0.70 $\mu g.g^{-1}$ which is similar to the Ontario mean for that year. The 1982 averages are based on 131 samples of Ontario wheat and 17 samples of Québec wheat.

In 1982 a larger number of samples of western wheat were also included in the DON survey. No detectable levels of vomitoxin were found in 114 samples of hard red spring wheat collected from the prairie provinces. Eighteen samples of Alberta red winter wheat and three samples of soft white spring wheat from Saskatchewan also showed no detectable DON levels. Eight of 31 samples of Alberta soft white wheat contained vomitoxin in the range of 0.02 to 0.07 $\mu g.g^{-1}$.

Extensive feeding trials with livestock and poultry indicated that swine can tolerate dietary levels up to 2 $\mu g.g^{-1}$ of DON without serious ill effects other than slight decreases in feed intake and weight gains (Friend et al. 1982; Young et al. 1983b, c). Poultry and dairy cattle appear to be more tolerant than swine (Moran et al. 1982; Hulan and Proudfoot 1982; Hamilton et al. 1983). In contrast to the results of the feeding studies, livestock producers have reported feed

refusal, vomiting and serious illness at very low DON levels (Côté et al. 1984). One can only conclude that the animals involved were very sensitive to DON ingestion, possibly because of strain differences or nutritional, health or environmental stresses, or that other mycotoxins not yet identified might have been present. Alternatively, mycotoxin interactions could have played a role in the adverse effects seen in the livestock.

In Canada, ergot-infested grain brought to a country elevator normally will be downgraded or rejected. Heavily infested grain may be diverted for special cleaning, often at terminal elevators. There is usually a market for ergot sclerotia. Elevator operators or growers may choose, however, to blend contaminated grain with clean grain to achieve higher grade status. Grains destined for export or domestic milling are inspected by the Canadian Grain Commission, which establishes tolerance levels for each grade and type of grain (Seaman 1980). Levels of ergot contamination in pedigreed and commercial classes of seed grains and in animal feed are regulated by Agriculture Canada. In feed, for example, ergot content may not exceed 0.1% by weight. Although grain moving through commercial channels in Canada should pose little or no ergot problem in flour and feed, the use of screenings (in which ergot levels are often high) for feed (e.g. as pellets) could cause problems in livestock and poultry. The on-farm use or inter-farm trade of uncleaned, contaminated feed grain is probably the most likely potential source of ergot problems in feed (Young 1981a, b; Young and Chen 1982). However, litigation involving commercial sources of ergot-infested feed has occurred in recent years in both western and eastern Canada.

Recently, an aflatoxin survey was conducted jointly by Agriculture Canada and Health and Welfare Canada on commercial dairy feeds offered for sale in Canada. One hundred complete dairy feed samples collected from across Canada for routine product monitoring were analyzed by the Health Protection Branch (HPB) Laboratory in Toronto for aflatoxins B_1, B_2, G_1 and G_2. No positive samples were identified.

Studies have also reported the extent and nature of fungal infections across Canada (Scott et al. 1970, 1972; Neish and Cohen 1981; Davis et al. 1982; Seaman 1982; Neish et al. 1983; Abramson et al. 1983a). However, there is still a great deal that we do not know about the relationships between mycotoxin contamination of Canadian feedstuffs and adverse effects in livestock and poultry. With the possible exception of DON, well designed mycotoxin surveys on Canadian feeds and feedstuffs are quite scarce, even though molds capable of producing various mycotoxins are known to be present in Canada. It is quite probable that significant advances in animal nutrition in the near future will be made by increasing our knowledge of the presence of mycotoxins and our ability to detoxify or avoid such compounds. The need for additional survey data in order to determine exposure to these compounds is evident. Mycotoxins, such as T-2 toxin, ochratoxin, zearalenone, ergot alkaloids and possibly DON, may be involved in some

cases of animal illness (Harwig and Munro 1975); the data suggest that the cause-effect relationships are indeed complex and involve other factors which are as yet poorly defined. In contrast to mycotoxins ingested as contaminants in feedstuffs, at Nappan, Nova Scotia, researchers have identified fungal metabolites of soil-borne fungi associated with "ill thrift" in livestock; these findings are discussed in Section 5.1.3.

2.2 MYCOTOXINS IN FOODS AND FOODSTUFFS (B.C. Williams)

The Field Operations Directorate, HPB, Health and Welfare Canada, performs both periodic and ongoing monitoring programs to determine if, and at what levels, various undesirable mycotoxins are present in foods offered for sale in Canada. The monitoring programs are based on non-biased samples and are designed to collect specific information on a cross-section of appropriate foods. In addition to the monitoring programs, compliance-oriented programs are also conducted as a means of ensuring that foods offered for sale comply with the requirements of the Food and Drugs Act and Regulations. Samples obtained for the compliance programs are usually biased.

2.2.1 PATULIN IN APPLE JUICE

During fiscal year 1977-78, HPB monitored apple juice from all major processors in Canada. Samples consisted of juice prepared directly from apples as well as juice prepared from apple concentrate (some of which had been imported). The HPB laboratory identified the limit of detection for patulin to be 11 $\mu g.L^{-1}$ at that time.

Of the 72 samples analyzed, 37% did not contain detectable levels of patulin. The average level in the remaining samples was 56.5 $\mu g.L^{-1}$. The highest level was 115 $\mu g.L^{-1}$ (confirmed).

During the survey it was observed that apple juice prepared from concentrate contained lower levels of patulin than juice prepared directly from fresh apples.

Following a five-month storage period, duplicate samples from nine of the original lots of juice were analyzed. The average concentration of patulin in samples from these particular lots decreased from 94 $\mu g.L^{-1}$ to 17.5 $\mu g.L^{-1}$.

2.2.2 ZEARALENONE

Between 1978 and 1980, the Field Operations Directorate, HPB, monitored 144 samples of imported and domestic dried corn and corn products destined for use as food, to determine the presence of zearalenone. The minimum level of detection reported by the HPB laboratory was 30 $\mu g.kg^{-1}$.

The results of the survey indicated that only one of 61 imported samples, 1.6%, contained detectable levels of zearalenone (200 $\mu g.kg^{-1}$). In contrast, 23 of the 81 domestic samples, 28.4%, contained detectable levels of zearalenone ranging from 13 to 475 $\mu g.kg^{-1}$, with an average level of 96.7 $\mu g.kg^{-1}$.

2.2.3 OCHRATOXIN A

During the period 1976-1978, HPB monitored 315 samples of various products (including wheat, oats, rye, corn, barley, soya beans, almonds, rice, malt, flax and buckwheat) for the presence of ochratoxin A. The detection limit was reported to be 2.5 $\mu g.kg^{-1}$. Only six samples were found to contain detectable levels of ochratoxin A, ranging from 3.0 to 8 $\mu g.kg^{-1}$.

Following the above study, in 1979 the HPB analyzed 84 samples of dried peas and beans together with 24 samples of processed cheese. Only one sample (imported dry beans) contained a detectable level of ochratoxin A (21 $\mu g.kg^{-1}$).

2.2.4 AFLATOXIN

(A) In an effort to determine the levels of aflatoxin present in peanut butter, the Field Operations Directorate, HPB, conducts an ongoing monitoring survey of various lots produced by all manufacturers in Canada, as well as of major imported brands. From April 1975 to March 1982, a total of 2477 non-biased samples of peanut butter were analyzed for aflatoxin. Only 1% of the samples were found to exceed the established tolerance of 15 $\mu g.kg^{-1}$. The following tabulation indicates the ranges of total aflatoxin for the 1% unsatisfactory group (only):

Total samples	15-30 $\mu g.kg^{-1}$	31-60 $\mu g.kg^{-1}$	61-90 $\mu g.kg^{-1}$	$\geq$90 $\mu g.kg^{-1}$
25	18	3	2	2

(B) In addition to the above monitoring program, the HPB also conducts a compliance program for the purpose of identifying those foods that may contain excessive levels of aflatoxin (followed by appropriate compliance action). The high priority foods are Brazil nuts, pistachio nuts, peanuts and pumpkin seed.

(C) In 1979-1980 HPB monitored 60 samples of imported cheese to determine the content of aflatoxin M_1 (a hydroxylated metabolite of aflatoxin B_1). The results of the survey indicated that 57 of 60 samples contained $\leq$0.26 $\mu g.kg^{-1}$ of aflatoxin M_1. The 3 remaining samples were found to contain 0.37, 0.55 and 0.89 $\mu g.kg^{-1}$. Seven of the samples that contained $\leq$0.26 $\mu g.kg^{-1}$ had measurable levels of aflatoxin M_1 ranging from 0.06 $\mu g.kg^{-1}$ to 0.25 $\mu g.kg^{-1}$.

2.2.5 DEOXYNIVALENOL (DON, VOMITOXIN)

Since August 1980 the HPB, together with Agriculture Canada, has been actively engaged in sampling and analyzing raw grains and finished foods to determine the presence of DON. During this period wheat crops from across Canada and dried corn from southwestern Ontario have been monitored in an attempt to determine the incidence and levels of DON.

Wheat

(i) Western Canada

Findings for the past four crop years indicate that western hard spring wheat has been relatively free of DON. Levels slightly in excess of the detectable level of 0.02 $\mu g.g^{-1}$ have been observed in only a very small portion of the samples analyzed.

In 1981 detectable levels of DON were observed in 50% of the samples of soft spring wheat produced in a limited area of Alberta. However, the majority of these samples contained ≤ 0.1 $\mu g.g^{-1}$. A similar survey in 1982 reported that only 5 of 33 specimens had detectable levels. The highest level of DON reported was 0.065 $\mu g.g^{-1}$.

(ii) Ontario

Trace levels of DON were detected in the majority of samples of Ontario soft winter wheat between 1980 and 1982. In 1981 the average level was 0.18 $\mu g.g^{-1}$, while in 1982 the average level was 0.74 $\mu g.g^{-1}$. In 1983 only a very limited number of samples exceeded 0.1 $\mu g.g^{-1}$.

(iii) Québec

Deoxynivalenol was detected in 1980 Québec hard spring wheat, intended for food use, at an average level of 0.5 $\mu g.g^{-1}$. In 1981 the entire Québec wheat crop was diverted to animal feed use. In 1982 the levels found in wheat intended for human consumption ranged from ≤ 0.005 to 0.372 $\mu g.g^{-1}$. In 1983 the majority of samples of wheat intended for human consumption contained ≤ 0.03 $\mu g.g^{-1}$.

(iv) Atlantic Provinces

Wheat grown for food use in Nova Scotia and New Brunswick was found to be relatively free of DON between 1980 and 1983. Only two samples had reported levels exceeding 0.1 $\mu g.g^{-1}$. During the same period DON appears to have been much more prevalent in the wheat grown on Prince Edward Island. The levels reported by HPB range from 0.06 to 1.59 $\mu g.g^{-1}$ in wheat intended for human consumption.

Dried Corn

Samples of dried corn from southwestern Ontario, intended for use in the processing of food, were monitored for DON in the fall of 1981, 1982 and 1983. The average levels of DON were 0.33 $\mu g.g^{-1}$, 0.22 $\mu g.g^{-1}$ and 0.17 $\mu g.g^{-1}$, respectively, for the 3 years.

Processed Foods

In addition to the surveys carried out on raw grains following harvest, the HPB has also monitored some finished food products to determine the presence of DON. Average DON levels found are shown in Table 2-2 (Scott 1984c).

Table 2-2. Deoxynivalenol (DON) in some wheat foods (procured 1982-83, imports included) (Scott 1984c).

Food	No. Samples Analyzed	DON, average ($\mu g.g^{-1}$)[a]
Wheat breakfast cereals	36	0.086
Wheat flour	43	0.40
Bran	14	0.17
Bread (including buns)	21	0.080
Cookies/biscuits	35	0.12
Crackers	20	0.27
Baby cereals (mixed)	30	0.043

[a] Product basis.

3.0 MYCOTOXIN PRODUCTION IN THE FIELD

3.1 MYCOLOGY

3.1.1 FUSARIUM (G. Neish)

Fusarium species can cause diseases of plants and can produce toxins that affect mankind either directly or indirectly, through their effects on economically important species of animals and plants (Palti 1978; Nelson et al. 1981). Consequently, the fusaria have been the subjects of thousands of investigations, and a significant part of this effort has been directed to the important task of delimiting taxa within the genus (Toussoun and Nelson 1975). Nonetheless, partly as a result of differences respecting species concepts, and partly as a result of the morphological and physiological diversity of the fusaria, there is presently no universally accepted taxonomic system for this genus. Thus obvious, and apparently simple, questions concerning the number of known taxa, and the distribution and potential for toxin production of the various taxa, cannot be answered with precision. Coupled with frequent misdeterminations of taxa, the present taxonomic confusion has unquestionably impeded the development of our knowledge of the fusaria and their toxins (Hesseltine 1977; Smalley et al. 1977; Marasas et al. 1984).

Fortunately, there is reason to hope that there will soon be a satisfactory solution to this problem. Recent important treatments of Fusarium taxonomy (Booth 1971, 1981; Gerlach and Nirenberg 1982; Burgess and Liddell 1983; Nelson et al. 1983) differ in detail but they are all based on Wollenweber's seminal contributions to this subject (Wollenweber and Reinking 1935). In addition, they all recognize the value of the International Code of Botanical Nomenclature, the value of the conidiogenous cell as an important taxonomic character for certain taxa, and the value of Snyder and Hansen's (1940) contribution to the taxonomy of the section Elegans (Fusarium oxysporum Schlecht.). Concomitant with this trend toward agreement on taxonomic and nomenclatural principles has been increased recognition of the need to develop further internationally accessible reference collections of cultures. While more progress is needed in this area, significant advances have been achieved in recent years at organizations such as the Commonwealth Mycological Institute (Booth 1971), the American Type Culture Collection (Jong and Davis 1978), the Fusarium Research Center at Pennsylvania State University (Fisher et al. 1982; Marasas et al. 1984) and elsewhere. Thus the basic thrust of recent work on Fusarium taxonomy is toward increased agreement on species concepts that can be verified by the examination of living cultures maintained by methods that reduce the risk of mutation (e.g. cryopreservation, lyophilization).

The number of Fusarium species likely to be of concern as natural sources of mycotoxins in food produced in Canada is probably less than a dozen (Table 3-1). This conclusion is based on the

Table 3-1. Fusarium species of greatest potential concern as sources of mycotoxins in Canadian grains.

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1. F. acuminatum Ellis & Everh.[α]
2. F. avenaceum (Fr.) Sacc.
3. F. culmorum (W.G. Smith) Sacc.[β,γ]
4. F. equiseti (Corda) Sacc.[β]
5. F. graminearum Schwabe[β,γ]
6. F. moniliforme Sheldon
7. F. oxysporum Schlecht. emend. Snyder & Hansen
8. F. poae (Peck) Wollenw.[γ]
9. F. sporotrichioides Sherbakoff[γ]
10. F. subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas
11. F. tricinctum (Corda) Sacc.

[α] Nomenclatuure and species concepts in accordance with Nelson et al. (1983)

[β,γ] Species for which isolates of Canadian origin have been reported to produce in vitro zearalenone (β) or one more trichothecenes (γ). For references, see Neish et al. (1982, 1983) and Miller et al. (1983a).

assumption that concerns about zootoxic Fusarium metabolites will continue to be focussed on contaminated grains, and that the taxa most commonly associated with grains will also be the most probable sources of these toxins (Neish et al. 1982). However, even if these assumptions are rejected - Harwig et al. (1979), for example, have shown that F. sulphureum Schlecht. can produce trichothecenes after inoculation into tomatoes and trichothecenes have recently been detected in Fusarium infected potato tubers (Lafont et al. 1983; El-Banna et al. 1984) - several species can still be excluded as sources of concern either because they probably do not occur in Canada (e.g. F. decemcellulare Brick), or because they are unlikely to occur on foodstuffs that will be consumed by man or domestic animals (e.g. F. aquaeductuum (Radlk. & Rabenh.) Lagerh.).

In Canada, the Fusarium toxins of greatest concern at present are DON and zearalenone because it has been demonstrated repeatedly that they occur as natural contaminants of grains in this country (Trenholm et al. 1981b; Scott et al. 1981; Sutton 1982; Neish et al. 1983; Scott 1984c). (See also Sections 2.1, 2.2.5 and 6.3.2.) Several Fusarium species have been reported to produce zearalenone (Caldwell et al. 1970; Ishii et al. 1974; Hacking et al. 1976) and DON production has been attributed to at least four Fusarium species (Vesonder et al. 1981a, b). Both toxins can be produced simultaneously by Canadian isolates of F. culmorum and F. graminearum (Vesonder et al. 1981b; Neish and Cohen 1981; Neish et al. 1983; Greenhalgh et al. 1983; Miller et al. 1983a; Cohen and Neish 1983, unpublished observations on F. culmorum isolates from barley grown in Alberta), but so far it would appear that field occurrences of these toxins in Canada and the United States can be attributed solely to population Group 2 (sensu Francis and Burgess 1977) isolates of F. graminearum (teleomorph: Gibberella zeae (Schw.) Petch). However, it is difficult to confirm this. Records of zearalenone and DON production have been attributed to Fusarium roseum (Pathre and Mirocha 1979) in the sense of Snyder and Hansen (1945), a name that includes not only F. graminearum, but also F. culmorum and numerous other taxa. Also, it is possible that records of mycotoxin production previously attributed to either F. graminearum or F. culmorum should, in fact, be attributed to the recently described species F. crookwellense Burgess, Nelson and Toussoun (Burgess et al. 1982).

T-2 toxin and diacetoxyscirpenol (DAS) have been reported to occur naturally in Canada (see Abramson et al. 1983a) but the Fusarium species that presumably produced these toxins was/were not identified. Present evidence suggests that F. sporotrichioides is the most probable potential source of T-2 toxin in Canadian grains and hay (Scott et al. 1980; Davis et al. 1982; Neish et al. 1982; Marasas et al. 1984;) but, in the absence of direct proof, other species, including F. graminearum (Miller et al. 1983a), cannot be ruled out. Canadian isolates of both F. poae and F. graminearum have been reported to produce DAS (Neish et al. 1982; Miller et al. 1983a).

Further studies are needed on the occurrence, distribution and taxonomy of toxigenic fusaria in Canada with particular emphasis on their capacity to produce toxins in commodities in which they are likely to occur and which are also likely to be consumed by animals or man.

3.1.2 ALTERNARIA (G. Neish)

Alternaria species are widely distributed in nature and may be saprotrophic or parasitic (Ellis 1971, 1976). Some species, such as A. alternata (Fr.) Keissler (= A. tenuis C.G. Nees) and A. tenuissima (Kunze: Fr.) Wiltshire, are cosmopolitan and plurivorous whereas others, such as A. longipes (Ellis & Everh.) Mason (associated with Nicotiana), A. solani Sorauer (associated with Solanum) and A. citri Ellis & Pierce (associated with Citrus), are limited to certain host plants.

Alternaria species are known to elaborate at least seven mycotoxins (Chu 1981) and the potential impact of these toxins on human and animal health has been reviewed recently by Scott and Stolz (1980) and by Stinson et al. (1980). Information presently available suggests that no Alternaria species can be ruled out a priori as a source of one or more of these toxins (Kinoshita et al. 1972; Stinson et al. 1980, 1981).

In North America, while there has been some interest in toxin production by the host-limited Alternaria species, including those listed above (Doupnik and Sobers 1968; Stinson et al. 1980, 1981), most attention has been focussed on the toxigenic potential of A. alternata (Meronuck et al. 1972; Davis et al. 1977; Sauer et al. 1978; Harwig et al. 1979; Stinson et al. 1980). Harwig et al. (1979) studied Canadian isolates of A. alternata and showed that they could produce in vitro alternariol, alternariol methyl ether and tenuazonic acid. These fungi also produced tenuazonic acid and alternariol methyl ether after being grown in ripe tomatoes. A. alternata is commonly isolated from grains and Alternaria toxins have been found in weathered grain sorghum in Kansas (Seitz et al. 1975; Sauer et al. 1978), but there are as yet no reports of these toxins in Canadian grains or other agricultural commodities available in Canada, except for tomato paste (Scott and Kanhere 1980). However, numerous Alternaria species have been reported from a wide variety of substrates in Canada (Conners 1967) and this fact, coupled with the potentially harmful effects of their toxins, suggests that this is an area that requires further investigation.

3.1.3 CLAVICEPS (W.L. Seaman)

In Canada the ergot disease of grains and grasses is caused by the fungus Claviceps purpurea (Fr.) Tul.; all cultivars of the cultivated grains and grasses and most weed and native grasses are

susceptible to C. purpurea. Other species of Claviceps occur on wild rice and on grasslike rushes (Juncus spp.) and sedges (species of Carex and Eleocharis).

Three aspects of the life cycle of this floret-infecting fungus are important epidemiologically: a sclerotial state, in which the fungus overwinters in the field or in stored grain; an ascospore state, which provides primary inoculum for infection in spring and early summer; and a conidial state, by which secondary infection from plant to plant occurs during the growing season.

Mycotoxins occur in the hard, purplish sclerotia produced by the fungus in infected florets. Sclerotia may fall to the surface of the soil or may be harvested with the grain. Those that overwinter on the surface or in the upper few cm of soil, or that are introduced into a field with infected seed, may germinate under favorable conditions in spring or early summer. A stroma develops from the sclerotium and is borne on a stalk (up to ca. 4 cm long), which extends the spore-bearing stroma above the soil surface; ascospores are released from perithecia formed within the stroma and are dispersed by air currents. An ascospore contacting an open floret of a susceptible host produces hyphae, which penetrate the ovary, killing the host tissues. Within a few days of infection, conidia are formed on the surface of the ovary and exude from the floret in a sugary matrix commonly called honeydew. Conidia may be spread to nearby florets on the same spike in droplets of honeydew dissolved in water from rain or heavy dew, or they may be carried further to nearby spikes by rain, wind and insects. In some florets, infections do not develop further, so that symptoms may consist only of sterile florets, resulting in reduced grain yield. Often, however, the mycelium grows extensively within the glumes and differentiates into a sclerotium which matures as the grain ripens. The sclerotium is usually purplish black with a characteristic dark outer rind and a grayish interior, and is often similar in shape and size to kernels of the host plant; however in some hosts, notably rye and timothy, the sclerotia are much larger than the kernels and protrude conspicuously from infected heads. The sclerotia may fall from the heads at maturity, or they may be harvested with the seed or, in pastures, they may be eaten by grazing animals. Whole sclerotia or large pieces of broken ones are usually conspicuous in harvested grain. Most are removed from lightly infested grain by modern seed cleaning equipment. However, the removal of sclerotia from heavily infested grain is difficult; it may not be possible to achieve grade standards with equipment used in commercial seed-cleaning operations. When ground with the grain for feed or flour, ergot particles are very difficult to detect except by microscopic, physiological, or chemical tests.

In grain fields, ergot infection is usually most prevalent among plants at the margin of the field, especially in fields bordered by uncut roadside or headland grasses. Within fields, ergot is found

frequently in volunteer plants from a previous crop (especially of rye), in thin stands, and on late-formed tillers. In many areas in Canada, ergot appears to be endemic in natural and seeded grasses, notably species of Bromus and Agropyron, from which inoculum moves to nearby fields. Infection of grasses is undoubtedly the chief means by which the fungus survives in cultivated areas; grasses along the shorelines of lakes and oceans, although usually well-removed from cultivated land, may also provide a source of perennation of the fungus (Krajnyk and Maun 1981).

Many grasses, and rye, are open pollinated and thus more often infected than wheat, barley and oats, which are normally self-pollinated. In self-pollinated grains, the florets are open for a very short period, during which they are exposed to Claviceps spores; once pollination occurs the ovary becomes less susceptible to infection. Cool, cloudy, wet weather favors development of the fungus and may also inhibit or delay pollination of host florets; thus outbreaks of ergot are frequently related to periods of wet weather at anthesis, especially in late-seeded fields or in those having many late-formed and thus late-flowering tillers. Conditions leading to floret sterility, for example the inappropriate use or timing of applications of growth regulators, such as herbicides, or of growth retardants that in some applications are also gameticides, may lead to floret sterility. Instances of such use have been associated with severe ergot infections, for example, in barley in eastern Ontario in 1983.

In certain areas of Canada problems with ergot occur almost annually in wheat, barley and rye. When weather conditions are favorable for infection, low levels of ergot occur annually throughout eastern Canada; in western Canada, problem areas seem to occur more frequently in eastern Saskatchewan and adjoining Manitoba and in east-central Alberta. In such areas ergot may flare up in common or durum wheat, barley and rye, depending on inoculum availability and weather conditions at the time of anthesis. In Alberta, ergot occurs more frequently in spring rye than in earlier flowering fall-seeded rye (Harper and Seaman 1980b). The impact of ergot on the yield and grading of rye is relatively small in most years (Harper and Seaman 1980a), but serious losses from the downgrading of contaminated barley and wheat occur sporadically in the prairie provinces. For example, in 1978 about 1% of western red spring wheat from certain areas of Saskatchewan was downgraded on account of ergot (Young 1979); in 1982 serious losses were experienced from ergot infection of spring wheat in the Calgary, Alberta area; and in 1983 locally severe infections occurred in southern Saskatchewan and in east-central Alberta (Harper 1984). Cultivars of triticale, a newly developed grain derived from intergeneric wheat and rye crosses, have been relatively susceptible to ergot, although problems with floret sterility are seen less frequently in newer cultivars. Triticale is being developed for both milling and feed uses but is not widely grown in Canada at present. Oats are rarely infected in the field. Historically, instances of ergot poisoning of humans have

been associated most frequently with infested rye flour; animal feeds may be contaminated with ergot from barley, wheat, triticale or rye; and occasionally animals have been affected by grazing on infected grasses in wet areas of pastures and rangeland.

Resistant cultivars and chemical treatments are not available for controlling ergot. The successful management of ergot in grain centers around farm practices that minimize the development of the disease. These include the use of sound, pedigreed seed, the cutting of grasses in infested areas before they head, the use of a nonsusceptible crop following one in which even small amounts of ergot are observed, the plowing of fields in which a severely infested crop occurs, the careful use of herbicides and other growth regulators, and the cleaning or suitable disposal of contaminated grain.

3.1.4 CHAETOMIUM (D. Brewer and A. Taylor)

There have been extensive taxonomic studies of the genus Chaetomium over many years. One of the most interesting studies, when considered in conjunction with mycotoxin production, is that of Dreyfuss (1975). In this study a large number of the species were grouped according to their morphological characteristics, nutritional requirements, etc. Four species-groups were circumscribed, with six additional groups outlined. Dreyfuss stated that many species are conglomerates of intermediate forms that tend to bind neighbouring species. Sekita et al. (1981) have pointed out that the species shown to produce chaetoglobosins are limited to the C. globosum - Gruppe sensu Dreyfuss, as are the chetomin-producing strains, with the exception of C. umbonatum which was not considered by Dreyfuss. Likewise, the species producing cochliodinol belong to the C. globosum - Gruppe. Isolates of C. aureum and C. trilaterale, both members of the C. aureum - Gruppe, produced oosporein. However, the toxic compound chaetochromin has been isolated from species from four different groups, leading Sekita et al. (1981) to suggest that this compound may be quite common. Udagawa (1979a) relegated Achaetomiella to synonomy with Chaetomium. Sekita et al. (1981) concluded that C. thielavioideum was identical to Achaetomiella virescens and that, based on morphological characteristics and identical patterns of metabolites, C. cellulolyticum is identical to C. thielavioideum.

Chaetomium spp. occur on a wide variety of agricultural products, including feedstuffs, and in soils; many of the species have worldwide distribution. Chetomin was established as the toxic agent in corn infested with C. globosum but this is the only case of a positive link between a mycotoxin produced by a species of Chaetomium and toxicity of feed to animals. The isolation of an oosporein-producing culture of C. trilaterale from moldy peanuts (Cole et al. 1974) and the reports (Brewer and Taylor 1978; Udagawa et al. 1979b; Sekita et al. 1981) of toxin-producing isolates of a wide range of Chaetomium spp.

make the investigation of isolates of Chaetomium from suspect feed and food products necessary, regardless of their species identity. The group to which the isolates belong may serve to indicate the type of toxin that may be produced.

3.1.5 TRICHODERMA (D. Brewer and A. Taylor)

The taxonomy of the genus Trichoderma is still somewhat confused. The tendency in early reports of isolations of Trichoderma from soils was to classify the isolates as T. viride, T. lignorum, or just Trichoderma sp. The study by Rifai (1969) was a major step in helping to clarify the situation. He described a number of species aggregates, but stated that each aggregate was composed of heterogeneous strains and that further study would probably divide these species aggregates into a number of species. Even with the relatively wide range of characteristics delimiting a species aggregate, it is still sometimes difficult, if not impossible, to determine to which aggregate an isolate belongs. The reports of isocyanide metabolites from a number of species aggregates of Trichoderma (Brewer and Taylor 1981; Fujiwara et al. 1982) and the possible implication of these compounds in the etiology of the ill-thrift syndrome emphasize the need for further study of the taxonomy of Trichoderma to aid in a detailed study of the ecology of the genus in soils. This is further emphasized by Okuda et al. (1982), who said that the production patterns by isolates of Trichoderma of these particular metabolites enabled them to establish sub-groupings within some species aggregates.

3.2 PLANT RESISTANCE AND ENVIRONMENTAL CONDITIONS (R.B. Hunter and J.C. Sutton)

Plant resistance to diseases has long been an objective of plant breeders. Obtaining germ plasm resistant to a given disease by means of selection, if possible, is the most direct approach to reducing or eliminating problems arising from the disease. The reduction or elimination of a disease organism capable of producing mycotoxins would have the effect of reducing or even eliminating concern about toxin formation. In general, research on mycotoxins has concentrated on symptoms expressed in animals, dose levels affecting animals, factors affecting growth, analytical techniques, chemical formulae and prospects for detoxification. It is only recently that research has been initiated on plant resistance to organisms producing toxins. In the United States the major emphasis has been on selection of corn germ plasm with lower levels of aflatoxin (Widstrom and Zuber 1983). In Canada the emphasis has been on resistance to Fusarium graminearum (Sutton 1982). Research is being conducted on both corn and wheat. For this reason the section that follows will deal with plant resistance to Fusarium graminearum.

Wheat head blight and corn ear rot, caused by F. graminearum, have common characteristics of paramount importance in resistance

breeding and other approaches to disease control. First, epidemics of these diseases appear to be completed in a single infection cycle (Sutton 1982) and are described as monocyclic. Second, the period when the host plants are receptive to the pathogen is relatively short. For both wheat and corn, the most receptive period is during and just after flowering. Finally, warm and wet conditions are required for disease outbreaks, and must coincide with the period of receptivity of the wheat or corn. For resistance breeding, techniques have been developed to aid in the screening of genotypes. There are genotypic differences in resistance to F. graminearum but no genotypes, either of corn or of wheat, have been found to be completely resistant.

3.2.1 THE INFECTION CYCLE

The principal inoculum source of F. graminearum is host debris, such as old stalks and ears of corn and stubble and debris of wheat and other small-grained cereals (Warren and Kommedahl 1973; Shurtleff 1980). Soil, seeds and infected crops are also potential sources of the fungus. Inoculum types include two kinds of spores, ascospores and macroconidia. The spores are dispersed in the air or carried by vectors amongst which adult picnic beetles (Glischrochilus quadrisignatus (Say)) and red-winged blackbirds (Agelaius phoenicius) have been identified, and some spores may be deposited on host plants (Sutton et al. 1980b; Tyler and Kannenberg 1980; Attwater and Busch 1983).

Spores deposited on wheat spikes germinate and grow initially on anthers, but the fungus later penetrates and colonizes the spikelets (Andersen 1948). Diseased spikelets become prematurely straw-colored or bleached in appearance. Under humid conditions superficial pink or whitish growth of the pathogen may be evident on the spikes and later on the grains.

F. graminearum deposited on corn ears grows initially on silks, anthers and floral bracts, and later on the kernels and cobs (Hesseltine and Bothast 1977; Naik and Busch 1978). Infection is promoted by wounds produced artificially (see Section 3.2.3.1) or by birds and insects (Sutton et al. 1980b; Sutton and Baliko 1981). Characteristic reddish or pinkish mold appears on the kernels. Disease normally progresses down the ear from the ear tip.

3.2.1.1 The Receptive Period

Wheat spikes are not receptive to F. graminearum prior to anthesis, but are receptive in the period from flowering to the soft dough stage (McKay and Loughnane 1945; Andersen 1948). The stage of peak receptivity differs among cultivars (Schroeder and Christensen 1963). Receptivity declines rapidly after the soft dough stage

(Andersen 1948; Strange and Smith 1971; Sutton 1982). Anthers stimulate growth of F. graminearum and promote infection of the spikes (Strange and Smith 1971). Emasculated spikelets rarely become infected.

Corn ears are most susceptible to F. graminearum shortly after the silks emerge and the susceptibility declines thereafter (Ullstrup 1970; Enerson and Hunter 1980a; Sutton and Baliko 1981).

The period of host receptivity is the critical time for outbreaks of head blight or ear rot. Weather factors favorable for production and dispersal of spores, growth on the host surface and infection must coincide with host receptivity for disease outbreaks to occur.

3.2.1.2 Weather Variables

Progress of almost all stages of the infection cycle requires temperatures of 15-35°C (optimum 25-32°C) (Liang et al. 1981; Sutton 1982). Free water or low water potentials are important for inoculum production and splashing rain aids in the dispersal of macroconidia (Ayers et al. 1975; Ye 1980; Sutton 1982). Persistent wetness on wheat spikes and corn ears for 48-60 h or more after initial exposure favors abundant infection (Sutton et al. 1980a; Sutton 1982).

3.2.2 RESISTANCE BREEDING IN WHEAT

3.2.2.1 Inoculation Techniques

Outbreaks of head blight in wheat are sporadic in nature. As a result, effective screening of wheat genotypes requires the development of an efficient inoculation technique that simulates natural infections. Early reports suggest the success of inoculation in differentiating between wheat genotypes for susceptibility to F. graminearum (Christensen and Stakman 1927). Andersen (1948) provided a detailed outline of an inoculation technique whereby the heads of potted plants were sprayed with a suspension of conidia and then placed in humidity tents to provide conditions suitable for infection. Hanson et al. (1950), working with field grown plants, sprayed heads with a suspension of spores and hyphae. The spraying was done in the late afternoon. To maintain the necessary high humidity environment for infection, the plants were subsequently sprayed with water and shaded. This inoculation technique provided a convenient method of screening wheat genotypes for resistance to F. graminearum in the field. Devaux (1982) has simplified the technique even more. By irrigating the inoculated plants for 10 seconds at half-hour intervals, he has eliminated the need for shading.

3.2.2.2 Resistance

Intervarietal differences in susceptibility to head blight were first reported by Arthur (1891). Since then many workers have demonstrated that genotypic variation exists (Hanson et al. 1950; Nakagawa 1960; Schroeder and Christensen 1963, Takegami and Sasai 1970). Resistance, however, appears to be relative; all wheats studied were infected to some degree (Christensen et al. 1929; Hanson et al. 1950; Schroeder and Christensen 1963). In an attempt to obtain more complete resistance in wheat, the International Center for the Improvement of Maize and Wheat (CIMMYT), Mexico, is screening for genes conferring resistance to F. graminearum that are found within species of Agropyron and Elymus (Anonymous 1982a). Christensen et al. (1929) found common wheat to be more resistant to head blight than were durum wheats. The nature of the inheritance of varietal reaction to head blight has not been well documented. Hanson et al. (1950) found evidence of genetic segregation in the F_3 generation. Nakagawa (1955) concluded that at least three pairs of factors governed head blight resistance. Results presented by Schroeder and Christensen (1963) indicate that resistance is due to a wide variety of factors which range from flowering habit to definite physiological defensive reactions of the host plant. Wheat lines Juanillo 97 and Toropi and some lines of triticale show markedly reduced incidence of head blight.

Miller et al. (1984) used a single isolate of F. graminearum to infect a number of head blight "resistant" and "susceptible" cultivars of wheat (9), rye (5) and triticale (6). They observed low concentrations of DON and ergosterol (a fungal metabolite used as a measure of biomass) in the resistant cultivars, and higher concentrations in the susceptible cultivars. The data suggested that "resistant" cultivars had greater resistance to hyphal invasion and had factors that prevented synthesis or promoted degradation of DON. The data suggested that ergosterol-to-DON ratios could be used to distinguish between the two types of cultivars.

3.2.3 RESISTANCE BREEDING IN CORN

3.2.3.1 Inoculation Techniques

Attempts have been made to develop inoculation techniques that would allow for screening and evaluation of corn germ plasm where natural infection is low. The technique of inserting toothpicks soaked in nutrient solution and colonized with F. graminearum into the silk channel at the tip of the ear effectively differentiated between resistant and susceptible genotypes (Ullstrup 1970; Enerson and Hunter 1980a; Sutton and Baliko 1981). Inoculation techniques in which wooden toothpicks overgrown with F. graminearum were plunged through the husks and into the ear were too severe (DeVay et al. 1957; Ullstrup 1970; Sutton and Baliko 1981). Injection of macroconidial suspension into the

ear tip also proved to be too severe (Ullstrup 1970). In contrast, inoculation of ears with suspensions of macroconidia applied to the ear tip of the silk channel without injury to the host produced infection levels too low for differentiating between genotypes (Koehler 1959; Ullstrup 1970; Sutton and Baliko 1981).

3.2.3.2 Resistance

Breeding corn for resistance to Fusarium ear rot has been suggested (Hooker 1956; DeVay et al. 1957; Koehler 1959; Ullstrup 1970). Genotype differences for susceptibility to ear rot have been clearly demonstrated (DeVay et al. 1957; Ullstrup 1970; Loesch et al. 1976; Enerson and Hunter 1980a, c; Cullen et al. 1981). Resistance to Fusarium ear rot is thought to be conditioned by many genes (Cabulea et al. 1977; Ullstrup 1977; Enerson and Hunter 1980b), implying that there is a predominantly synergistic relationship between host and pathogen effects. An examination of the interactions between corn hybrid and F. graminearum strain suggests that strains differ in pathogenicity and in ability to produce vomitoxin and zearalenone (Atlin et al. 1983). However, there was insufficient interaction between corn hybrids and strains to warrant using more than one strain for resistance breeding. A recent paper by Mesterházy (1982) comes to the opposite conclusion.

There are several characteristics of maize that are known to affect the establishment and development of Fusarium ear rot. Upright ears and those with tight husk coverage at harvest are more susceptible than pendant ears and those with loose husks (Koehler 1959; Enerson and Hunter 1980c). Wimmer (1978) also suggests that plants with rudimentary ears and sterile ear tips are more susceptible.

3.3 TRANSLOCATION WITHIN THE PLANT (J.C. Young)

Among the various concerns raised by the presence of mycotoxins in agricultural commodities is the possibility of fungus and toxins appearing in parts of plants remote from the site of initial infection. In a preliminary study, Sutton et al. (1976) sought evidence for translocation of zearalenone in corn plants colonized by F. graminearum. They found that the fungus colonized only the inoculated portions (ear or stalk) of the plant, that zearalenone was not translocated from the ear to the stem, and that evidence for stem to ear translocation of zearalenone was only circumstantial. After ear inoculation of field corn with F. graminearum, Young and Miller (1984) observed the fungus, the metabolite ergosterol, and the mycotoxin DON in the ear, husk, axial stem and stalk. Zearalenone and 15-acetyl-deoxynivalenol were detected only in the ear and husk. Lee et al. (1980) noted highly variable levels of aflatoxin and Aspergillus flavus spores in individual corn kernels within a given ear; thus interkernel translocation appears unlikely for this mycotoxin. Aflatoxin can,

however, be translocated from a hydroponic culture medium into seedling roots and then into the stem and leaves (Llewellyn *et al*. 1982). Jarvis *et al*. (1981) located a shrub that can absorb certain macrocyclic trichothecenes from soil and translocate them to other parts of the plant.

4.0 MYCOTOXIN PRODUCTION DURING STORAGE

(D. Abramson and J.T. Mills)

4.1 OCCURRENCE OF MYCOTOXINS AND ASSOCIATED FUNGI IN STORED PRODUCTS

Mycotoxin contamination of feeds and foods has been scrutinized during the past twenty years from two main aspects; first, the identification of mycotoxins posing significant health risks to humans and animals, and, second, the study of their occurrence in major crops, animal feeds, and food products. Hesseltine (1974), Scott (1973, 1978a) and others have reviewed the literature on the nature and extent of mycotoxin contamination in foods and feeds. Current knowledge of mycotoxin occurrence indicates that contamination patterns in cereals and their products differ with geographical location and bioclimatic zone (for example, Bottalico 1979; Tuite 1979; Hökby et al. 1979; de Campos et al. 1980; Connole et al. 1981). Mycotoxin problems reported to occur in field and storage in the diverse bioclimatic zones of Canada have been summarized by Andrews et al. (1981). Previous studies of mycotoxin development in stored products have dealt mainly with investigating the natural occurrence of mycotoxin, and preventing their development by the use of controlled-composition atmospheres and chemical agents.

Shotwell et al. (1975) mapped the aflatoxin and zearalenone levels around a naturally-occurring hot spot in an Illinois corn bin, and identified Aspergillus flavus on some contaminated samples. Lillehoj et al. (1975) investigated the aflatoxins and mycoflora in the discolored surface corn samples from a Missouri bin and found aflatoxin B_1 levels of 400 $\mu g.kg^{-1}$, A. flavus and other fungi including A. terreus, A. niger, A. glaucus gr. spp., and Penicillium spp. Trenk and Hartman (1970) stored naturally-infected corn at different moisture contents and temperatures and studied aflatoxin production and mycofloral occurrence. That study demonstrated aflatoxin B_1 production by local strains of A. flavus at moisture contents of 17.5% and above and at temperatures of 24°C or above. Aspergillus, Penicillium, Fusarium, Chaetomium species and other fungi were also present.

The use of defined atmospheres to control mycotoxin development was investigated by Wilson and co-workers. Corn, freshly-harvested at 24% moisture content (MC) or remoistened to 19.6% MC, inoculated with A. flavus and stored at 27°C for four weeks developed, in most trials, aflatoxin levels of 2 $\mu g.kg^{-1}$ in the following controlled atmospheres: CO_2 14.2% + O_2 5% + N_2 84.4%; CO_2 0.7% + O_2 0.3% + N_2 99%; CO_2 65.3% + O_2 4.2% + N_2 30.5% (Wilson and Jay 1975). Peanuts remoistened to 16.7% MC and naturally infected with A. flavus were similarly stored and developed levels of 21 $\mu g.kg^{-1}$ or less of aflatoxin B_1 in the following controlled atmospheres: CO_2 14.7% + CO 0.05% + O_2 3% + N_2 84%; CO_2 12.3% + CO 2.7% + O_2 0.4% + N_2 83.7% (Wilson and Jay 1976). Freshly-harvested corn, at 18.8% MC and naturally infected, was stored for 109 days at 24% MC in an atmosphere of average

composition CO_2 15.3% + O_2 1.6%. Only 15 $\mu g.kg^{-1}$ aflatoxin B_1 was detected after this time; infection rates were A. flavus, 47%, Fusarium moniliforme, 22%, and Penicillium sp., 27% (Wilson et al. 1977). More recently Fabbri et al. (1980) stored soft wheat inoculated with A. flavus at 18.5% MC and 32°C for 21 days under a nitrogen atmosphere, and found less than 1 $\mu g.kg^{-1}$ aflatoxin B_1.

The use of chemical agents to control mycotoxin formation has also been studied. As an example, Bothast et al. (1976) stored two naturally-infected lots of corn (27% MC) in bins for seven months after treating one lot with isobutyric acid and the other with propionic-acetic acids. In the former treatment, up to 857 $\mu g.kg^{-1}$ aflatoxin B_1 was found; A. flavus, A. fumigatus, A. niger, Absidia sp., Penicillium sp. and Scopulariopsis brevicaulis were present. In the latter treatment no aflatoxins were found; A. fumigatus, Absidia sp., Penicillium sp., and S. brevicaulis were present. No samples from either treatment contained ochratoxins or zearalenone. Other chemical treatments such as dichlorvos (Rao and Harein 1973) and o-vanillin (Bilgrami et al. 1982) showed some inhibition of aflatoxin formation, but the residues may affect the suitability of the crops as feed.

Canadian studies on mycotoxins and their associated fungi in stored products are summarized in Table 4-1.

4.2 MYCOLOGY: GROWTH AND DEVELOPMENT OF TOXIGENIC FUNGI IN STORED PRODUCTS

The process of fungal growth consists of primary and secondary metabolic stages. Toxins may be produced at the secondary metabolic stage. The two stages may follow each other closely or may be separated by a prolonged interval. They may occur on crops in the field, in storage, or in both locales (Figure 4-1). Thus, stored crops may contain toxins which developed while the crop was standing in the field and/or toxins developed in storage (Mills 1982).

For growth and development, each toxigenic fungus has particular moisture, temperature, air and substrate requirements, which may differ from those required for toxin production. The growth requirements of the main toxigenic fungal genera occurring in Canadian stored products are discussed below.

(a) Aspergillus and Penicillium

Seeds entering storage may be contaminated by propagules of species of post-harvest (storage) Aspergillus and Penicillium. Dormant conidia originating from soil particles, plant debris and combine residues may occur on seed surfaces (Flannigan 1978). More rarely, dormant mycelium may be present under the seed pericarp (Christensen and Kaufmann 1969).

Table 4-1. Mycotoxins and associated fungi in stored products in Canada 1972-83.

Commodity	Mycotoxin(s) Maximum Levels, $\mu g.kg^{-1}$	Associated Fungi[a]	Sample Source or Features	History of Storage	Reference	Remarks
wheat	ochratoxin A, 27 000 citrinin, 80 000	P. viridicatum P. cyclopium	associated with human lung problems	unknown, but stored damp	Scott et al. 1971	
rye	ochratoxin A, 720 citrinin, 960	P. viridicatum	"	"	"	
oats	ochratoxin A, 3000 citrinin, 10 000	"	"	"	"	
wheat & wild oats	ochratoxin A, 70	P. viridicatum P. cyclopium	" "	" "	" "	
oats & barley	ochratoxin A, 22 000 citrinin, 60 000	P. viridicatum	"	"	"	
horse feed	ochratoxin A, 20	P. cyclopium A. ostianus	mycotoxicosis	unknown	"	composition undefined
dairy ration	ochratoxin A, 530	P. viridicatum P. cyclopium	mycotoxicosis	"	"	"
white beans	ochratoxin A, 2100	P. viridicatum P. cyclopium P. palitans	moldiness	"	"	
peanuts	ochratoxin A, 4900	P. viridicatum P. cyclopium	"	"	"	
wheat	sterigmato-cystin, 300	P. viridicatum A. versicolor	associated with human lung problems	unknown, but stored damp	"	
wheat	ochratoxin A, 700	P. cyclopium	granary storage study	21% MC, 20 wk.	Abramson et al. 1980	wheat I 14-25°C
barley	ochratoxin A, 3900	"	"	"	"	barley I 14-25°C
flaxseed	aflatoxins B_1 + B_2, 460	A. flavus	water-damaged in 1979 Red River flood	unknown	Mills and Abramson 1981	

Table 4-1. (Cont'd)

Commodity	Mycotoxin(s) Maximum Levels, $\mu g.kg^{-1}$	Associated Fungi[a]	Sample Source or Features	History of Storage	Reference	Remarks
wheat	ochratoxin A, 41	P. cyclopium	laboratory storage study	20.5% MC, 10 wk, 22°C	Abramson et al. 1982	
wheat	ochratoxin A, 8	"	"	20.5% MC 10 wk, 15°C	"	
poultry feed	ochratoxin A, 140	A. ochraceus Penicillium spp.	mycotoxicosis	unknown	Abramson et al. 1983a	30% barley content
oats & barley	T-2 toxin, 2500 diacetoxy-scirpenol, 800, ochratoxin A, 380	P. cyclopium Fusarium spp.	mycotoxicosis	bin inundated with rain seepage	"	sample contained live insects
poultry feed	sterigmato-cystin, 2300	A. glaucus gr. spp.	mycotoxicosis	unknown	"	
barley	ochratoxin A, 48	P. cyclopium	mycotoxicosis	high moisture storage	"	
wheat, oats & barley	ochratoxin A, 5900	P. cyclopium A. ochraceus	moldiness	unknown	"	
barley	sterigmato-cystin, 800	A. versicolor	granary storage study	16% MC, 66 wk	Aoramson et al. 1983b	barley T, 3-22°C
barley	sterigmato-cystin, 3450 ochratoxin A, 240	P. cyclopium A. versicolor	"	20% MC, 66 wk	"	barley T, 3-26°C

[a] Aspergillus flavus Link; A. glaucus group species: mainly Eurotium amstelodami Mang., E. repens de Bary, and E. rubrum Konig et al.; A. ochraceus Wilhelm; A. ostianus Wehmer, A. versicolor (Vuill.) Tiraboschi; Fusarium spp.: not determined; Penicillium cyclopium = P. verrucosum var. cyclopium (Westling) Samson, Stolk & Hadlok; P. palitans = P. verrucosum var. melanochlorum Samson, Stolk & Hadlok; P. viridicatum = P. verrucosum Dierckx var. verrucosum.

T = temperature range during storage.

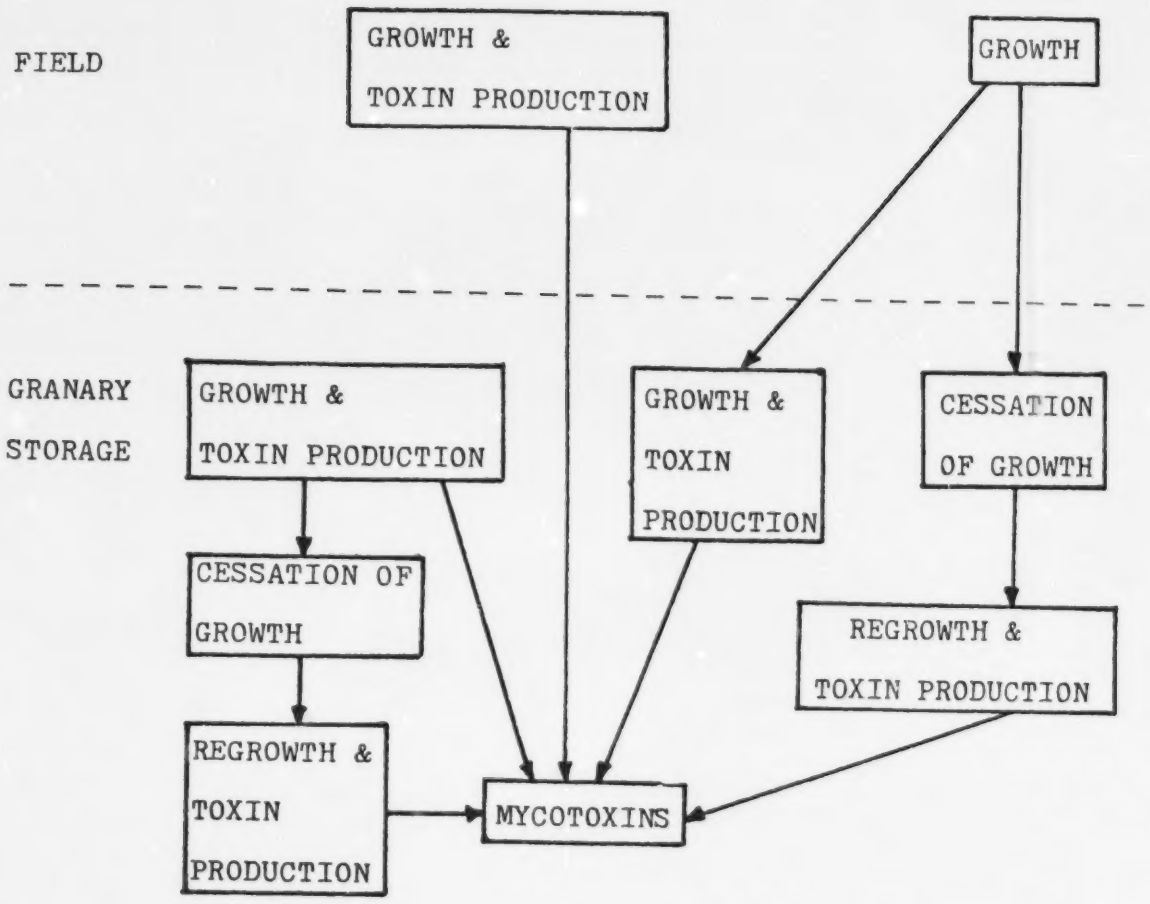

Figure 4-1. Origin and development of toxigenic fungi in stored products.

Table 4-2. Minimum relative humidity (RH) and time lapse in days required for observed conidial germination of toxigenic species of Aspergillus and Penicillium at 4 to 37°C (Mislivec et al. 1975).

Species		Temperature, °C						
		37	30	26	16	12	8	4
A. flavus	RH	83	81	86	90	95	-[α]	--
	Days	9	14	5	14	9	--	--
A. versicolor	RH	81	81	81	83	90	--	--
	Days	20	12	12	15	15	--	--
P. cyclopium	RH	--	83	81	81	83	90	90
	Days	--	8	22	25	33	33	33
P. viridicatum	RH	90	86	81	81	83	90	95
	Days	17	17	10	24	35	35	35

[α] Conidial germination was not observed after 35 days.

In Canada, several mycotoxicoses resulting from the ingestion of stored products have been associated with the occurrence of Aspergillus versicolor, P. cyclopium and P. viridicatum. The RH-temperature requirements for conidial germination of these fungi have been studied by Mislivec et al. (1975), and, together with similar data for A. flavus, are summarized in Table 4-2. The minimum RH requirements for conidial germination in the four species was 81%, a level approximating that obtained in equilibrium with 16.0-16.5% MC wheat and corn (Christensen and Kaufmann 1969). Conidia of A. versicolor did not germinate below 12°C whereas those of P. cyclopium and P. viridicatum were able to germinate slowly below 2°C. P. cyclopium and P. viridicatum have different upper temperature limits for germination; P. viridicatum grows at 35°C, but P. cyclopium grows poorly if at all at that temperature (Mislivec and Tuite 1970; Mislivec et al. 1975). P. cyclopium appears similar to P. viridicatum, but the colonies are blue rather than green. Conditions required for fungal growth and toxin production by Aspergillus and Penicillium have been investigated both in the laboratory and in granary storage studies.

(i) Laboratory studies

Development of ochratoxin A on wheat and barley has been extensively studied. Northolt et al. (1979) investigated the effects of water activity (a_w) on the growth of, and ochratoxin A production by, strains of A. ochraceus, P. cyclopium and P. viridicatum on sterilized ground barley. The temperature range for growth of P. viridicatum was 0-31°C at 0.95 a_w, and 12-24°C for ochratoxin A production. Optimum a_w for mold growth as well as ochratoxin A production was at 0.97 a_w. On malt extract agar, the germination times for P. viridicatum under optimum conditions were 0.5-2.0 days, increasing to 12-14 days under unfavorable conditions. Northolt (1979) also measured growth of toxigenic strains on agar by determining mycelial dry weight and colony volume.

Harwig and Chen (1974) used nonsterilized whole or ground wheat or barley adjusted to various moisture contents, inoculated with strains of P. viridicatum, and incubated at 5, 12 or 25°C for 28 days. Mold counts and growth observations were made. At 0.90-0.93 a_w, high concentrations of ochratoxin A were obtained together with abundant P. viridicatum. Below this a_w, lower levels of ochratoxin A were associated with P. viridicatum and native microflora. Viable barley and wheat seed was less prone to ochratoxin A development than heat-killed seed, although mold counts were the same.

Abramson et al. (1982) used naturally-infected non-sterilized wheat adjusted to 20.5% MC and incubated at 15 and 22°C for 70 days. Production of ochratoxin A was accompanied by development of P. cyclopium and increased fungal propagule counts.

Mills and Abramson (1982) assessed the ochratoxigenic potential of Penicillium species isolated from stored rapeseed and cereals in western Canada on ground barley. Of their 34 isolates, 3 isolates of P. cyclopium and 4 of P. chrysogenum from 5 lots of rapeseed produced ochratoxin A. This was the first reported occurrence of P. chrysogenum as a producer of ochratoxin A.

(ii) Granary studies

Fungal growth and toxin development have been investigated in both short- and long-term studies. The bin investigations of Lillehoj et al. (1975) and of Shotwell et al. (1975) were described previously (Section 4.1). In both studies, A. flavus was the predominant fungus. Mills and Abramson (1981) investigated microfloral changes occurring in farm-stored grains affected by flooding of the Red River in Manitoba. Results of laboratory flood-damage simulation studies with wheat, rapeseed and flaxseed generally confirmed those found in the field - the only mycotoxins found in either study were aflatoxins B_1 at 426 $\mu g.kg^{-1}$ and B_2 at 34 $\mu g.kg^{-1}$ in flaxseed taken from a partially filled bin affected by floodwaters. When disturbed, the flaxseed released clouds of A. flavus spores.

In more detailed long-term granary studies, Seitz et al. (1982) studied fungal growth as measured by ergosterol content of grain, loss of dry matter, and levels of aflatoxin during the bin storage of high-moisture corn. The corn was stored in two 100-bu bins and aerated from the bottom. The fungal invasion of kernels was found to be highest at the top, and lowest at the bottom of the bins. Abramson et al. (1980, 1983b) monitored changes occurring within 0.018 m^3 (0.5-bu) lots of moist cereals placed within bulk oats. In the first study (Abramson et al. 1980), 0.018 m^3 (0.5-bu) lots of barley, wheat and oats were adjusted to 20.5% MC and stored for 20 weeks. Ochratoxin A production occurred primarily in the barley, and was associated with strains of P. cyclopium. In the second study, barley at 16% and 20% MC was stored for 66 weeks (Abramson et al. 1983b). Ochratoxin A and sterigmatocystin were produced at 20% MC by 20 weeks. Ochratoxin A production was associated with P. cyclopium, and sterigmatocystin with A. versicolor and A. glaucus group species. In a third study (Abramson et al. 1984), wheat at 15 and 19% MC was stored for 60 weeks. No mycotoxins were detected at either of the moisture content levels, and it is thought that approximately 19% MC may represent a critical moisture limit for mycotoxin formation in granary stored wheat, given the indigenous mycoflora.

(b) Fusarium

Grain entering storage may have been invaded by toxigenic strains of F. culmorum, F. graminearum, F. poae, F. sporotrichioides, or F. tricinctum under high humidity conditions in the field (Section 3.1.1). Further development may occur in grains stored at excessive

humidity, e.g. exposed corn cobs in cribs (Diener 1976). Critical moisture content levels for development of Fusarium spp. vary between 22.2 and 33.0% in cereals (Martin and Gilman 1976). Growth of Fusarium spp. in soil is generally restricted by minimum requirements of 88-91% RH (Palti 1978). Fusarium spp. may die out after drying or storage of seeds, but the toxins persist (Joffe 1963). Most fusaria have temperature optima for mycelial growth around 20-30°C, but are capable of development at 12-20°C. Senescent tissues of wheat, barley, oats, rye and corn are particularly susceptible to invasion by Fusarium (Palti 1978).

Conditions required for Fusarium growth and mycotoxin development have been investigated mainly in the laboratory on a small scale. Sherwood and Peberdy (1974) found that sterilized corn, wheat, barley and oats inoculated with F. graminearum were susceptible to fungal establishment at MC above 18%, even at 7°C. At 15-18% MC, risk of fungal establishment increased at 18-25°C, and maximum zearalenone production was observed at 12 and 18°C. Vesonder et al. (1982) studied growth and DON production by F. graminearum strain, Northern Regional Research Laboratory (NRRL) 5883, and F. roseum NRRL 6101 on trichothecene-free cracked corn adjusted to 30% MC. Both strains grew equally well; optimum DON production occurred at 26-30°C after 40-41 days incubation. In a field-and-laboratory study, Gross and Robb (1975) sprayed a barley crop with spores of a zearalenone-producing strain of F. culmorum. The barley was stored at 34% MC and 25°C for three weeks, and at 15°C thereafter, conditions thought optimal for zearalenone production. None of this toxin was found until after ten weeks, indicating that considerable delay may occur between infection and appearance of mycotoxins.

4.3 ECOLOGICAL PARAMETERS OF MYCOTOXIN PRODUCTION IN STORAGE

Mycotoxin production in the field depends on factors described previously (Section 3.0). Production of mycotoxins at harvest time is affected by physical factors (e.g. moisture, temperature, rapidity of drying, rewetting, relative humidity) and the degree of exposure of the crop to mechanical injury and extensive spore inoculation (Hesseltine 1974, 1976). These physical factors plus subsequent storage conditions might facilitate further toxin development. However, mycotoxin contamination is frequently absent before storage, and arises as a result of improper storage practices.

The spoilage of stored crops, fungal growth and mycotoxin formation result from the interaction of many factors, some of which are depicted in Figures 4-2A, 4-2B and 4-2C. At the beginning of storage (Figure 4-2A) the crop, or "substrate", is contaminated by fungal spores from the air, other grain or dockage. Mechanical damage from harvesting or from the action of insects may damage the outer seed coat and facilitate infection. Insects, through their movements, may also aid in spreading the inoculum. Subsequent fungal growth (Figure 4-2B)

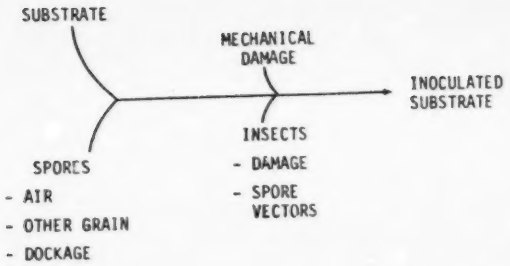

Fig. 4-2A.

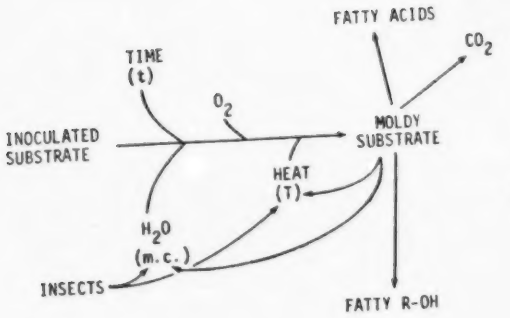

Fig. 4-2B.

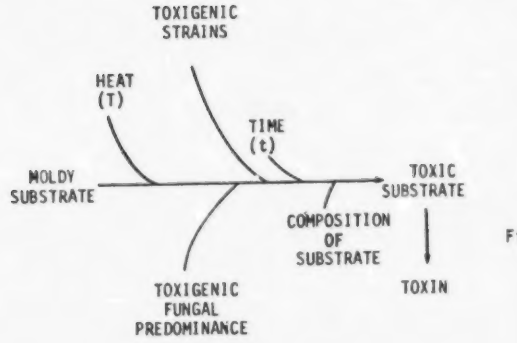

Fig. 4-2C.

Figure 4-2. Aspects of mycotoxin production in post-harvest storage.

depends on available oxygen, time, heat and moisture. The last two factors may be augmented by insect activity as well as by the metabolism of the fungi themselves. Sufficient moisture to trigger the spoilage process may be present because of rain seepage, the melting of wind-driven snow, or moisture migration and condensation on the bin walls (Muir 1973). Chemical by-products of the moldy substrate, e.g. free fatty acids, CO_2, and various fatty alcohols which partially constitute fungal odors, are indicated as possible early-warning signs of impending mycotoxin formation. Subsequent development of the toxin (Figure 4-2C) depends on the presence and predominance of toxigenic fungal strains, sufficient heat for further fungal development, a substrate of suitable chemical composition for that particular fungus, and sufficient time for fungal metabolism to enter into its secondary phase.

The salient features of studies on mycotoxins in granary and laboratory storage experiments have been summarized in Table 4-1. Abramson et al. (1980, 1982, 1983b) investigated factors possibly connected with mycotoxin production. The following effects, in addition to changes in mycotoxin levels and fungal infection with time, were monitored: increases in MC, temperature, propagule count, CO_2 and free fatty acid; decreases in O_2 and seed germination; and the appearance of fungal odors. The fungal volatiles 3-methyl-1-butanol, 1-octanol, and 3-octanone (Kaminski et al. 1974) were detected in both the granary and the laboratory storage experiments.

From the studies so far, there emerge three main patterns of contamination that indicate potential problems:

(i) barley -- Penicillium -- ochratoxin A

(ii) wheat -- Penicillium -- ochratoxin A

(iii) barley -- A. versicolor -- sterigmatocystin

Although crops would not normally be stored at the MCs used in these experiments, moisture pockets may develop accidentally, as described above. These spoilt crops are later encountered in animal poisoning cases as mycotoxin-contaminated feed samples.

To evaluate the risk of mycotoxin formation in storage, it is necessary to examine cereals, oilseeds and other agricultural commodities, crop by crop, in the particular bioclimatic zone of interest. Differing climatic environments, regional occurrence of different toxigenic fungal strains, variations in crop species and cultivars, and bin aeration and management practices underline the need for further storage studies.

5.0 NUTRITIONAL AND TOXICOLOGICAL EFFECTS IN LIVESTOCK

5.1 TOXICOLOGY (H.B. Schiefer)

5.1.1 INTRODUCTION

As a first step in the safety evaluation of chemicals entering, either intentionally or unintentionally, into the feed and food supply, it has become general practice to ascertain the dose levels necessary to induce mortality or disease. The selection of the manner in which the test material is to be administered should reflect the potential route of exposure. With respect to mycotoxins, the literature shows that almost every possible portal of entry has been employed experimentally, the major routes being oral, dermal, intraperitoneal and intravenous. While many routes of application provide useful information on the effects of these mycotoxins, e.g. comparisons of rate and extent of absorption, metabolism, biotransformation and excretion, it is probably more appropriate to highlight the oral route for livestock. Consequently, the following discussion will focus on this route, which is well in agreement with the requirements set out for food safety assessment (Food Safety Council 1978).

The occurrence of mycotoxins in Canada has been discussed in Chapter 2.0. In contrast to those reports of mycotoxins in food and feed, only very limited information is available on actual disease outbreaks in Canada which can be more or less clearly associated with mycotoxins (Bristol and Djurickovic 1971; Greenway and Puls 1976; Puls and Greenway 1976; Davis *et al.* 1982; Abramson *et al.* 1983a; Voigt 1983). There are various reasons for this lack of information:

(i) Mycotoxins, by their nature, only rarely produce unique disease features.

(ii) By the time a disease is suspected to be linked to a mycotoxin, the spoiled feed may no longer be available for analysis; thus the causal relationship cannot be documented (Schiefer and O'Ferrall 1981).

(iii) Analytical means for detection of mycotoxins are not readily accessible to producers.

(iv) Because many mycotoxins exert subtle effects on the immune system, other diseases (bacterial, viral, parasitic) may develop secondarily, and are then diagnosed as the primary cause of death or poor performance. Thus, postmortem statistics do not record the actual occurrence of mycotoxicoses.

(v) Usually combinations of mycotoxins are produced in spoiled feed. Not all mycotoxins can be identified in every

analytical laboratory. Therefore the mycotoxins detected during analysis may not be the only ones actually present.

(vi) Some mycotoxins exert adverse effects at very low levels of administration. Currently available analytical methodologies are, in most instances, capable of detecting the mycotoxins at trace levels where, apparently, they have not yet affected nutritional and health parameters.

(vii) Representative samples may not be taken or properly stored. As a consequence analysis may not reflect levels present in the original feed.

Feuell (1966) listed the following diagnostic features of outbreaks of mycotoxicosis:

(i) the diseases are neither infectious nor contagious;

(ii) treatment with drugs (e.g. antibiotics) has little effect on the course of the disease;

(iii) in field outbreaks the trouble is often seasonal, as particular climatic events may favor toxin production by the mold;

(iv) careful study indicates association with a specific feedstuff (or foodstuff in the case of human mycotoxicosis); and

(v) examination of the suspected feedstuff reveals signs of fungal activity.

5.1.2 THE MYCOTOXICOSES OF CONCERN TO THE CANADIAN LIVESTOCK INDUSTRY

The mycotoxicoses of concern to the Canadian livestock industry (see Harwig and Munro 1975) include zearalenone, trichothecenes, ergot alkaloids and ochratoxin. Dicoumarol, produced by sweet clover in response to fungal invasion, also causes problems to the livestock industry. Brief descriptions of the toxins and their effects on farm animals are discussed below.

5.1.2.1 ZEARALENONE MYCOTOXICOSIS

SYNONYMS: F-2 toxicosis, Fusarium-toxicosis, vulvovaginitis (misleading terms describing symptoms)

SPECIES MAINLY AFFECTED: Swine.

BIOLOGICAL EFFECTS: Zearalenone, an estrogenic metabolite, produces hyperestrogenic effects. In addition, zearalenone has anabolic

properties which result in weight gain. This discovery has resulted in the marketing of a derivative, zearalanol ("Ralgro"), used in beef production. In a two-generation study with rats, the lesion attributable to zearalenone was increased medullary trabeculation of the femur in animals given the high dose of 10.0 $mg.kg^{-1}$ body weight (b.w.) (Becci et al. 1982a). No tumorigenic effects were noted when zearalenone was fed to rats for a lifetime (Becci et al. 1982b).

PHARMACOKINETICS: Studies with swine have shown that the toxin is absorbed into the blood stream within minutes after oral dosing. Low levels of free zearalenone and high levels of both conjugated and α-zearalenone persist in the blood for up to seven hours after dosing. Both conjugated and α-zearalenone can be detected in the urine within seven hours of ingestion of the toxin (Farnworth and Trenholm 1981, 1983).

CLINICAL SIGNS: The hallmark of zearalenone-toxicosis is the estrogenic effect, particularly visible in prepuberal gilts (Bristol and Djurickovic 1971). Within 4-7 days after zearalenone is ingested, hyperemia and edema of the vulva, vaginal prolapse and sometimes rectal prolapse may occur. The term "vulvovaginitis" is often used to describe the condition. The mammary glands and nipples may become enlarged. Mature sows show little or no effects, but lactating sows, secreting the toxin with the milk, often have nursing piglets that show enlargement of the vulva. There are reports that zearalenone can inhibit fetal development (Long et al. 1982) and it is suspected that zearalenone can cause abortion, stillbirth, or abnormal gait in newborn animals ("splay-leg"). Fertility problems are not uncommon because of the estrogenic effects on the ovaries (constant estrus, pseudo-pregnancy) (Kurtz and Mirocha 1978; Chang et al. 1979; Long et al. 1983). Immature male swine will have initially accelerated sexual maturation, followed by reduced spermatogenesis (Vanyi and Szeky 1980) and lack of libido; mature boars are not adversely affected (Ruhr et al. 1983).

PATHOLOGICAL FINDINGS: The vaginal and uterine tissues of gilts show signs of hyperplasia and cellular proliferation similar to those resulting from physiologic estrogenic stimulation. The ovaries are hypoplastic with no evidence of corpora lutea formation. The mammary glands show epithelial hyperplasia and edema.

LEVELS OF ZEARALENONE REQUIRED TO INDUCE SYMPTOMS: Concentrations of 1 to 5 $\mu g.g^{-1}$ of zearalenone in the feed are sufficient to produce clinical signs in swine (Kurtz and Mirocha 1978; Young et al. 1981, 1982). A single dose as low as 3.5 $mg.kg^{-1}$ BW produced estrogenic symptoms in young female pigs (Farnworth and Trenholm 1981). Boars fed 40 $\mu g.g^{-1}$ zearalenone had reduced libido scores (Berger et al. 198[illegible]. Zearalenone is often found together with DON in infested corn, thus complicating the clinical disease.

OTHER SPECIES AFFECTED: Cattle, sheep, poultry. Calves have been reported to show earlier sexual maturity, and dairy cows were

reported to have vaginitis, prolonged estrus and infertility (Palti 1978). In poultry, edema of the vent, infertility of males and cystic ovaries have been described (Palti 1978) but, in general, poultry are thought to be much less sensitive than swine to zearalenone (Chi et al. 1980a, b; Allen et al. 1981, 1983).

5.1.2.2 DICOUMAROL TOXICOSIS

SYNONYM: "Sweet clover poisoning". Dicoumarol is not a true mycotoxin. It is produced by the sweet clover in response to fungal infection. There are also other plants capable of providing the basic substrate which is needed by the fungi to convert the substrate to dicoumarol.

SPECIES MAINLY AFFECTED: Cattle.

BIOLOGICAL EFFECTS: Dicoumarol acts as a vitamin K antagonist and interferes with the synthesis of prothrombin and the blood coagulation factors VII, IX and X. The exact mechanism by which dicoumarol acts remains unknown.

CLINICAL SIGNS: When spoiled sweet clover hay, in which dicoumarol has been formed, is ingested, extensive hemorrhages occur in subcutaneous, muscular, and serosal tissue, which may cause pain and stiffness (reluctance to move). The hemorrhages occur at sites easily subjected to pressure or trauma. After giving birth, intrauterine hemorrhages may be observed in cows fed dicoumarol contaminated hay and their calves may be weakened or die. When the loss of blood is severe, signs of hemorrhage and anemia appear: the animal is weak and the heart rate increases (Radostits et al. 1980). The disease is generally known by the name "Sweet clover poisoning" because of the assumption that the toxic principle resides solely in sweet clover.

PATHOLOGICAL FINDINGS: Hemorrhages and hematomas in different stages of organization and resorption may be found in various locations of the body.

LEVELS OF DICOUMAROL REQUIRED TO INDUCE SYMPTOMS: Not known.

OTHER SPECIES AFFECTED: Occasionally, there have been unconfirmed reports of dicoumarol poisoning in horses. Care must be taken to avoid confusion with reports on accidental poisoning of various animal species with the rodenticide, Warfarin, a derivative of dicoumarol.

5.1.2.3 TRICHOTHECENE (NON-MACROCYCLIC) MYCOTOXICOSIS

SYNONYMS: Fusariotoxicosis, alimentary toxic aleukia in man, "Moldy corn poisoning" of cattle and swine, "Bean-Hulls poisoning" of horses.

SPECIES AFFECTED: All.

BIOLOGICAL EFFECTS: The trichothecenes elicit a wide range of effects, such as reduced feed palatability (feed refusal); emesis; cutaneous irritation (dermatitis, stomatitis); gastrointestinal irritation (gastroenteritis, intestinal villous atrophy); cytotoxicity = radiomimetic injury (damage to dividing cells in intestine, bone marrow, lymph nodes, thymus and spleen, leading to leukopenia or pancytopenia); neurotoxicity (tremor, convulsions, wing paresis in birds); teratogenicity; as well as a number of suspected effects (abortion, infertility, hepatotoxicity). For further references see Hayes (1979), Hayes et al. (1980), Hayes and Schiefer (1980, 1982), Ueno (1980), Schiefer (1983) and Rousseaux and Schiefer (1983).

PHARMACOKINETICS: The trichothecenes affect protein and DNA synthesis, and cell membranes (reversible alteration of polyribosomes). The toxins are quickly eliminated in feces and urine (Matsumoto et al. 1978; Nakano et al. 1979; Robison et al. 1979a), but not all toxins or their metabolites may be eliminated within a few days. Excretion through milk (Robison et al. 1979b; Yoshizawa et al. 1981) and eggs (Chi et al. 1978) may occur. HT-2 toxin appears to be the most commonly recognized metabolite after application of T-2 toxin, but this metabolite is almost as potent as T-2 toxin.

CLINICAL FINDINGS: The wide range of biological activities (see above) results in a very varied expression of clinical signs. Besides being accompanied by the presence of DON and neurological signs, most natural outbreaks have been associated with hemorrhagic diathesis syndromes and necrotizing lesions around the mouth and in the upper alimentary tract. When investigated, lymphopenia and neutrophilia have been observed on hematological examination.

PATHOLOGICAL FINDINGS:

(i) GROSS FINDINGS: Necrotizing and hyperplastic inflammation of the upper alimentary tract (mouth, esophagus, gizzard, stomach), atrophy or reduction of size of lymphatic organs (e.g. spleen, lymph nodes), and inactive bone marrow are the most commonly reported features. Hemorrhages in various organs may be present.

(ii) HISTOLOGICAL FINDINGS: The necrotizing and hyperplastic inflammation of the upper alimentary tract can be confirmed; a diagnosis of villous atrophy in the small intestine can be made only if one is sure that there are no other causes involved; the germinal centres of

lymph follicles in spleen and lymph nodes are necrotic; the bone marrow is hypocellular.

The cutaneous irritancy is the basis for the semiquantitative and semiqualitative skin bioassay (Hayes and Schiefer 1979).

There are often other, secondary lesions (see below).

LEVELS OF NON-MACROCYCLIC TRICHOTHECENES REQUIRED TO INDUCE DISEASE: The science of determining acceptable levels is in its infancy, mainly because experiments with pure trichothecenes (such as T-2 toxin, diacetoxyscirpenol (DAS), DON and fusarenon-X) have not been able to reproduce all the clinical features observed in natural "fusariotoxicoses". For dairy cattle, 2 $\mu g.g^{-1}$ of T-2 toxin in the diet caused chronic to subacute lesions and death in 20% of the animals (Hsu et al. 1972); 0.1 mg.kg $b.w.^{-1}$ caused death in 65 days (Buck and Osweiler 1976). Swine have been reported to refuse DON-contaminated corn containing 0.7 $\mu g.g^{-1}$ (Mirocha et al. 1976) or 1 $\mu g.g^{-1}$ (Vesonder et al. 1973). Feeding trials indicated that decreased weight gain and feed consumption can occur in swine at dietary levels of 0.5-1.0 $\mu g.g^{-1}$ of DON (Friend et al. 1982). At these levels no serious indications of illness (complete feed refusal, vomiting, hemorrhaging) were observed in swine, poultry and dairy cattle (Friend et al. 1983a; Hamilton et al. 1983; Moran et al. 1982; Trenholm et al. 1983). Chickens are apparently more tolerant to DON (Huff et al. 1981; Moran et al. 1982). Cereal grain contaminated with DON is often also contaminated with zearalenone (Pathre and Mirocha 1979), which adds to the difficulties of clinical recognition of disease symptoms.

OTHER LESIONS OR EFFECTS: Since trichothecenes have immunosuppressive effects (Buening et al. 1982; Friend et al. 1983b, c, d), secondary bacterial, viral and parasitic infections are not uncommon. Bronchopneumonia is particularly common. An endemic disease of the skeletal system of humans in East Siberia, China and Korea (Kashin Beck or Urov disease) is thought to be caused by trichothecenes (Palti 1978). A somewhat similar disease in chickens is referred to as "rickets in broilers" (Palti 1978).

5.1.2.4 TRICHOTHECENE (MACROCYCLIC) MYCOTOXICOSIS

SPECIES AFFECTED: All.

BIOLOGICAL EFFECTS: The biological effects are essentially the same as those produced by non-macrocyclic trichothecenes.

CLINICAL SIGNS: Stachybotryotoxicosis in animals is associated with the same cutaneous and mucocutaneous lesions, panleukopenia and hemorrhagic diathesis that are seen in non-macrocyclic trichothecene toxicosis, but death may occur in 2-12 days (Mirocha

1980). The disease has not been identified in Canada so far. Reports of occurrence of the disease have been restricted to the U.S.S.R, Eastern Europe, Finland, France, South Africa and New Zealand.

Myrotheciotoxicosis[α] causes death in 2-5 days, and there are indications that dendrodochiotoxicosis[β] caused death in a horse within hours after ingestion of 350-450 g of infested straw (Bridges 1978).

PATHOLOGICAL FINDINGS: Necrosis and inflammation of the digestive tract, hemorrhages in various organs and suppression of the hematopoietic system are the hallmarks of this disease.

LEVELS OF MACROCYCLIC TRICHOTHECENES REQUIRED TO INDUCE DISEASE: There are no published reports on undesirable levels of these mycotoxins, but it appears, from the field case descriptions, that the macrocyclic trichothecenes are more potent than the normocyclic ones.

5.1.2.5 ERGOT MYCOTOXICOSIS

SYNONYM: Ergotism.

SPECIES AFFECTED: All.

PHARMACOLOGY: Ergot sclerotia contain a variety of pharmacologically active substances, such as histamine, acetylcholine and alkaloids, either derived from lysergic acid or clavines. The alkaloids present in sclerotia differ greatly between individual sclerotia and geographical locations, but Young (1981a, b) and Young and Chen (1982) found that average values are quite similar for the various regions within Canada.

CLINICAL SIGNS: Nervous system disorders, tremor, convulsions, diarrhea, cold and insensitive limbs and necrosis of the extremities (gangrene) have all been reported. These symptoms are more likely to occur in cases of high doses of ergot alkaloids (Buck and Osweiler 1976; Mantle 1978; Christensen 1980).

SPECIAL PROBLEMS as a result of low doses of ergot alkaloids are not so easily identified. Besides reduced feed intake (Friend and MacIntyre 1970), abortion and stillbirth have been reported and agalactia in sows appears to occur rather frequently in association

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α - myrotheciotoxicosis: a disease of sheep grazing on rye straw infected with Myrothecium verrucaria in the U.S.S.R.

β - dendrodochiotoxicosis: a serious disease of horses in the Soviet Union caused by trichothecenes from Dendrodochium toxicum (Myrothecium roridum).

with ergot in feed. In chickens, which appear to be particularly sensitive, blackening of the comb, toes and beak are seen.

PATHOLOGICAL FINDINGS: Findings at necropsy are not very specific, unless gangrene is found. All other lesions can be caused by a plethora of conditions (Dignean et al. 1984).

LEVELS OF ERGOT CONTAMINATION IN THE DIET REQUIRED TO INDUCE SYMPTOMS:

(i) FOR CATTLE: 0.5% ergot in the diet causes reduced feed consumption and weight gain, but the no-effect dosage may well be below 0.1%, if one considers symptoms such as lameness, etc. to be ergot-related.

(ii) FOR SWINE: Sows fed 0.5 or 1.0% ergot have been reported to have almost no udder development, (Nordskog and Clark 1945), but in a recent experiment (Dignean et al. 1984), pregnant and nursing sows on diets containing up to 0.2% ergot had normal pregnancies and raised their litters normally.

(iii) FOR POULTRY: ≤0.8% in the diet.

(iv) OTHER LESIONS OR EFFECTS: Ergot alkaloids have effects on the endocrine system (prolactin, growth hormone, gonadotropins, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), insulin and steroidogenesis), but most of these observations have been made in laboratory animals. There are conflicting reports on the teratogenic potential of ergot alkaloids.

5.1.2.6 OCHRATOXIN MYCOTOXICOSIS

SYNONYM: Mycotoxic nephropathy.

SPECIES MAINLY AFFECTED: Swine, poultry.

BIOLOGICAL EFFECTS: Ochratoxin A damages mainly the proximal tubules of the kidneys; extrarenal effects are observed only after exposure to high levels, and include enteritis, necrosis of the lymphoid tissues, hepatic necrosis and fatty changes, and fetal malformation (Krogh 1976).

CLINICAL SIGNS: Ochratoxins are nephrotoxic and cause tubular degeneration accompanied or followed by interstitial fibrosis. Because of the short life of swine destined for slaughter, the renal lesions do not reach levels that can be diagnosed clinically. Polydipsia and polyuria are unnoticed under circumstances of hog raising and although clinico-pathological tests in experimental cases show that proteinuria, increased blood urea nitrogen and creatinine levels are present, these

features are not looked for under field conditions (Krogh 1976). In poultry, the principal signs are reduced weight gain, enteritis and dehydration (Peckham 1978).

PATHOLOGICAL FINDINGS: Kidneys of affected animals are grossly enlarged and pale. Histologically, tubular degeneration and interstitial fibrosis can be found. In addition to similar renal lesions, poultry often show fatty livers (Peckham 1978).

LEVELS OF OCHRATOXIN REQUIRED TO INDUCE DISEASE: 1 to 4 $\mu g.g^{-1}$ of ochratoxin in the diet of experimental swine caused lesions indistinguishable from those seen in natural cases. Dietary levels of 0.3 to 1.0 $\mu g.g^{-1}$ were sufficient to cause renal and hepatic lesions in poultry.

OTHER SPECIES AFFECTED: Ruminants (cattle, sheep) have been reported to develop renal lesions (Lloyd and Stahr 1979) and fetal death and abortion have been suspected to be caused by exposure to ochratoxin (Ribelin et al. 1978).

OTHER EFFECTS: Ochratoxins are said to have immunosuppressive effects, but 4 $\mu g.g^{-1}$ of ochratoxin A in the diet of mice did not cause depression of the immune response (Prior 1978; Prior and Sisodia 1982). Ochratoxin may affect the fertility and embryo viability of poultry (Prior et al. 1979). It may also be found in the tissues of animals ingesting contaminated food (Shreeve et al. 1979).

OCHRATOXIN AND OTHER MYCOTOXINS: Ochratoxins are frequently found together with citrinin. Citrinin causes lesions that are essentially indistinguishable from those caused by ochratoxin (Carlton 1980; Mehdi et al. 1981).

5.1.2.7 AFLATOXIN MYCOTOXICOSIS

SPECIES MAINLY AFFECTED: All. Mammalian: porcine ≥ bovine ≥ ovine. Avian: duck ≥ turkey ≥ goose ≥ chicken ≥ quail.

BIOLOGICAL EFFECTS: Aflatoxin has effects on cellular membranes, inhibits DNA-dependent RNA synthesis, and interferes with certain forms of enzyme induction. The mutagenic and carcinogenic effects are well known and have been extensively studied (Patterson 1983).

PHARMACOKINETICS: The aflatoxin molecule, which can be biodegraded in at least six ways (Patterson 1977) while retaining most of its toxicity, is excreted in conjugated forms in urine.

CLINICAL SIGNS: The clinical signs are non-specific and can be ascribed to any other type of toxicity or disease. Symptoms observed include: diarrhea, impaired growth, increased serum hepatic enzymes.

PATHOLOGICAL FINDINGS:

(i) ACUTE AFLATOXICOSIS: Grossly, livers of affected animals are enlarged and fatty; hemorrhages may be found in various parts of the body. Histologically, hepatic necrosis, fatty infiltration, and bile duct proliferation are the most outstanding findings.

(ii) CHRONIC AFLATOXICOSIS: Grossly, livers are enlarged, grey or tan colored and of firm consistency. Frequently, nodular hyperplasia or tumor formation occurs. The histologic findings are megalocytosis, bile duct proliferation, hepatic nodular hyperplasia and adenoma or carcinoma, as well as widespread fibrosis (cirrhosis). (Adapted from various chapters in Purchase 1974; Wyllie and Morehouse 1977, 1978; Rodricks et al. 1977; Moreau 1979)

LEVELS OF AFLATOXIN B_1 IN DIET REQUIRED TO INDUCE SYMPTOMS:

(i) FOR CATTLE: 0.7 to 1.0 $\mu g.g^{-1}$ induce chronic lesions. Death of calves can occur at levels of 2.2 $\mu g.g^{-1}$; 1.5 $\mu g.g^{-1}$ in dairy cows diets caused excretion of aflatoxin M in milk.

(ii) FOR SWINE (4-6 wks): $\geq$0.7 $\mu g.g^{-1}$ diet causes growth depression and liver damage. 0.3 $\mu g.g^{-1}$ appears to be the level at which no lesions occur (Miller et al. 1981).

(iii) FOR POULTRY (1 wk): 0.8 $\mu g.g^{-1}$ causes liver damage.

(iv) FOR DUCKLINGS: 0.30 $\mu g.g^{-1}$ causes death.

In general, it is suggested that feed not contain more than 20 µg aflatoxin B_1/kg.

The European Economic Community (EEC) tolerances for aflatoxin B_1 are (Krogh 1977, adapted from Buck and Osweiler 1976 and Wyllie and Morehouse 1977-1978):

For straight feed, mixed feed for cattle, sheep and goats:	50 $\mu g.kg^{-1}$
For mixed feeds for swine and poultry:	20 $\mu g.kg^{-1}$
For other feeds:	10 $\mu g.kg^{-1}$

OTHER LESIONS OR EFFECTS: Aflatoxins, known to be immunosuppressive agents, can be instrumental in the induction of secondary diseases. The often reported hemorrhagic diathesis symptoms in field cases may or may not be associated with aflatoxin, because moldy feed may contain other mycotoxins, in addition to aflatoxins. Carry-over into milk and tissues (Shreeve et al. 1979) occurs.

Sterigmatocystin, frequently found with aflatoxins, has a similar mode of action to aflatoxin, although it is less potent.

5.1.3 ILL-THRIFT OF SHEEP (D. Brewer and A. Taylor)

The term "ill-thrift" is used by the authors to describe poor growth of sheep which cannot be explained by parasitism, trace element imbalance or disease. A detailed study of ill-thrift of sheep as it occurs in Nova Scotia showed that the onset of poor growth was in the summer when there was apparently sufficient herbage to support growth of the animals (Brewer et al. 1971). Although there were no disease symptoms, there was a decrease in growth rate with a concomitant marked decrease in the bacterial count, both viable and total, in the rumen. Brewer et al. (1972b) suggested that ill-thrift may be due to the fungi in the soil capable of producing antibiotics and other metabolites, which were ingested by the sheep and thus affected rumen fermentation (Sections 1.12 to 1.13). In support of this suggestion it was shown that Trichoderma hamatum is a major component of the fungal flora of soils where ill-thrift occurs (Brewer and Taylor 1980, 1981) and that 50% of the isolates of this species produce isocyanide III (Figure 1-17). This metabolite, when fed to sheep at a daily oral dose level of 2.5 mg.kg b.w.$^{-1}$, did not produce any signs of toxicity, but did reduce the effective digestion of fodder by 13%, with a concomitant reduction in growth.

5.1.4 OTHER MYCOTOXICOSES, SO FAR NOT DIAGNOSED, THAT MIGHT OCCUR IN CANADA (H.B. Schiefer)

1) PITHOMYCOTOXICOSIS: The fungus, Pithomyces chartarum, along with its toxin, sporidesmin, has been reported in New Zealand and is the cause of so-called "Facial eczema". The toxin is hepatotoxic and causes a photodynamic dermatitis.

2) SLAFRAMINE MYCOTOXICOSIS: Excessive salivation and diarrhea have been reported in cattle, sheep and horses consuming red clover hay affected by "black patch disease".

3) RUBRATOXIN MYCOTOXICOSIS: Hepatotoxicity in swine, cattle and horses has been reported.

4) TREMORGENIC MYCOTOXINS: The clinical and pathological findings are tremors and convulsions, leading to death. The syndrome may affect calves, swine and sheep.

5) MONILIFORMIN MYCOTOXICOSIS: This Fusarium moniliforme toxin is known to cause ataxia and leukoencephalomalacia in Equidae.

6) "FESCUE FOOT" OF CATTLE is a poorly defined disease expressing itself as gangrene of the feet, similar to ergotism. The active principle is thought to be a trichothecene.

7) DIPLODIOSIS: The fungus, Diplodia maydis, may elaborate a toxin causing neurotoxicity, paralysis, ataxia, salivation and enteritis in cattle and sheep.

5.2 ANIMAL PRODUCTIVITY[α] (R.M.G. Hamilton)[β]

Livestock and poultry producers are well aware when acute mycotoxicosis occurs in their animals (cattle and swine) and birds (chickens and turkeys), because the toxic symptoms, as discussed in the previous section, are generally very pronounced, (5.1). The effects of chronic mycotoxicosis, especially at low or variable mycotoxin levels, may not be as readily seen by producers nor be diagnosed by a veterinarian because the changes that occur in such variables as feed consumption, feed efficiency, growth, mortality, morbidity, reproductive performance, egg production, egg and shell quality and resistance to secondary disease infections are small and not noticed (Pier et al. 1980; Smith 1982a). As a result, the financial losses may be as great with low-level chronic as with acute mycotoxicosis since the livestock or poultry producer may not be aware of the presence of the former in his animals or birds and thus will not have taken corrective action to resolve the problem.

In this presentation, discussion will be limited to the effects of DON, ochratoxin, T-2 toxin and zearalenone on cattle, swine and poultry because the results of a recent survey indicated that the ingestion of feeds or feedstuffs contaminated with these mycotoxins was the primary cause of mycotoxicosis in livestock and poultry in Canada (Andrews et al. 1981). Although aflatoxicosis causes serious losses in livestock (Lynch 1972; Pier et al. 1980) and poultry (Cavalheiro 1981) in some countries, aflatoxins are seldom found in feedstuffs used in Canada (Funnell 1979). Occasionally, reference will be made to aflatoxin, because extensive research has been done on this mycotoxin and its effect on livestock and poultry production.

5.2.1 FACTORS THAT INFLUENCE THE EFFECTS OF MYCOTOXINS ON LIVESTOCK AND POULTRY

The effects of mycotoxins in livestock and poultry are influenced by a number of factors besides dose level. The factors

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[α] A.R.C. Contribution No. 1275.

[β] Disclaimer: For personal reasons the author does not support Section 9.0 of this document.

which follow are based mainly on research with swine and poultry because of the paucity of this type of data for cattle.

1. Age of animal or bird. Young animals and birds are generally more sensitive to mycotoxins. Allcroft and Lewis (1963) reported reduced feed intake and growth, as well as death, of calves fed diets containing groundnut meal contaminated with aflatoxin; however, 3-year old heifers and cows 8-10 years old were not affected when fed diets containing the same meal. Lanza et al. (1980) found that for broiler chickens fed a diet containing 5 mg aflatoxin.kg diet^{-1} from hatching to 21 days of age, body weight was 137 g lower than for birds given the control diet. When these diets were fed to them between 21 and 42 days of age, the weights were only 6 g lower for the toxin-fed birds. The 7-day LD_{50} for broiler chicks was estimated by Huff et al. (1974) to be 2.14 and 3.60 mg.kg body weight^{-1} (b.w.) when the birds were given a single oral dose of ochratoxin A at 1 and 21 days of age, respectively. Chang et al. (1981) also found that the 7-day LD_{50} was higher when turkey poults were given a single oral dose of ochratoxin A at 21 days than at 1 day of age (7.84 vs 4.63 mg.kg b.w.$^{-1}$). The 10-day median lethal dose of orally administered T-2 toxin was found to be 5.14, 4.97 and 6.27 mg.kg b.w.$^{-1}$ for day-old broiler chickens, 8-week old broiler chickens and 24-week old Leghorn pullets, respectively (Chi et al. 1977).

2. Species of animal or bird. Marks and Bacon (1976) reported that diets containing corn infected by Fusarium graminearum and containing 25 or 100 mg zearalenone.kg^{-1} or purified zearalenone at these levels did not affect body weight, sexual maturity, egg and shell quality, or fertility and hatchability of eggs; egg production was significantly higher for hens given the zearalenone-containing diets. However, Young et al. (1981) found that feed intake, feed/gain ratio and body-weight gain were reduced when growing gilts (64 kg) were given diets containing up to 9 µg zearalenone.g^{-1} from corn inoculated with F. roseum. The effects of DON are also less pronounced on poultry than on swine. Results reported by Friend et al. (1982) indicate lower feed intake and daily body-weight gains of young growing gilts fed diets containing up to about 0.75 µg DON.g^{-1} from naturally-contaminated white winter wheat compared with animals fed a control diet that contained only trace amounts of DON. On the other hand, when Hamilton et al. (1983) fed Leghorn chicks, broiler chickens and turkey poults from 7 to 28 days of age diets containing similar DON levels from the same supply of wheat as that used by Friend et al. (1982), there were no differences in feed intake, feed/gain ratio or body-weight gain between the birds given the toxin-contaminated diets and those receiving control diets that contained no measurable toxin. Huff et al. (1981), Hulan and Proudfoot (1982) and Moran et al. (1982) also found that broiler chickens could tolerate high levels of DON before feed refusal, decreased growth or increased mortality occurred. Prior et al. (1976) found that the 7-day LD_{50}s for single oral doses of ochratoxin A for day-old White Leghorn chicks, turkey poults and Japanese quail were 3.4, 5.9 and 16.5 mg.kg b.w.$^{-1}$, respectively.

3. Sex of animal or bird. Prior et al. (1980) found that the growth of broiler chickens was reduced proportionally as the level of purified ochratoxin A in the diet increased, but the depression in growth was more marked and prolonged in males than in females. Results published by Friend et al. (1982) indicate that gilts (42 kg b.w.) consumed less feed and gained less weight than barrows (44 kg b.w.) when both sexes were fed for 49 days a diet containing 1.50 mg DON and 0.08 µg zearalenone.g^{-1} from naturally contaminated white winter wheat. Chi et al. (1980a) reported that female broiler chickens given diets containing about 50 µg purified zearalenone.g^{-1} did not grow as well as males fed the same diets.

4. Route of administration. Published data indicate that mycotoxins have been administered to experimental animals, particularly in toxicity studies, via several routes. The effects produced by a mycotoxin are influenced by the route used to administer it to experimental animals and birds. For example, Forsyth et al. (1977) estimated the minimum emetic dose of DON for 10-kg swine to be 0.1-0.3 mg.kg b.w.$^{-1}$ when administered orally and 0.05 mg.kg b.w.$^{-1}$ when injected intraperitoneally. Vomiting, hemorrhagic lesions in the intestines and metabolic disturbances have been described following acute parenteral administration of T-2 toxin or DAS to cattle (Weaver et al. 1978a), swine (Weaver et al. 1978a, b; Pearson 1978) and poultry (Weaver et al. 1978a). A hemorrhagic syndrome was not observed in cattle (Weaver et al. 1978a, d; Patterson et al. 1979) or swine (Pier et al. 1976) when T-2 toxin was given by chronic oral dosing or by feeding in diets. Ingestion is the principal route through which livestock and poultry are exposed to mycotoxins (Carlton and Tuite 1980).

Burditt et al. (1983a), using 7-day old, male Leghorn chicks from which feed or water had been withdrawn overnight, found that the addition to a chick starter diet of DAS in a filtrate of a F. roseum (NRRL 1181) culture (87 mg DAS.kg^{-1}) resulted in a 72% reduction in feed intake and replacement of the drinking water with the culture filtrate (335 µg DAS.mL) resulted in a 92% reduction of water intake over a 6-hour period of refeeding or rehydration; a filtrate of the culture medium had no effect on feed intake and caused an increase ($p \leq 0.05$) in water intake. In another study, Burditt et al. (1983b) concluded that the detection of a refusal factor or factors in 7-day-old chicks was more likely when the factor(s) was (were) presented in the drinking water rather than in the feed. Also, they found that T-2 toxin, DAS, ochratoxin A, and unknown factors from P. citrinum and A. ochraceus cause feed refusal in poultry.

5. Source of mycotoxin. In general, mycotoxins in naturally-contaminated feedingstuffs appear to be more potent, on the basis of equal quantity, than those that have been chemically purified. Speers et al. (1977) reported that feed intake and egg production was much lower for Leghorn hens fed a diet containing 16 µg T-2 toxin.g^{-1} from corn inoculated with F. tricinctum than for hens fed a diet containing the same level of purified T-2 toxin. Feed refusal in swine was found

to be much greater when the diet contained DON from naturally infected corn than when it had equal quantities of purified DON (Forsyth et al. 1977). Similar results were reported by Madsen et al. (1982a, b) who found that incorporating purified ochratoxin A (1.4 $\mu g.g^{-1}$) into the diet of growing castrated male swine (42 kg b.w.) had no influence on feed or water intake, whereas these variables were reduced significantly when the diet contained an equivalent quantity of the mycotoxin from naturally-contaminated barley; both diets contained 0.65 mg citrinin.kg^{-1}. One explanation given for the difference in response between purified and naturally-occurring mycotoxins was the possibility that other undefined factors were present in the naturally-contaminated feedstuffs (Forsyth et al. 1977; Madsen et al. 1982a, b). Another reason could be a difference in the availability of mycotoxins between purified and natural sources, but this postulate remains to be established.

6. Length of exposure. The effects on farm animals of ingesting some mycotoxins are transitory since there is an almost immediate decrease, for example, in feed intake and growth which later return to values similar to those obtained with control diets not containing mycotoxins. Friend et al. (1982) observed significantly lower average daily gains and daily feed intake for growing gilts during the first 3 days of receiving a diet with 0.75 μg DON.g^{-1} than for animals fed a control diet containing 0.05 μg DON.g^{-1}. However, there was no difference ($p \geq 0.05$) in the daily gain for days 3 to 21 of the experimental period between the swine fed the DON-containing and those fed the control diet. Choudhury et al. (1971) reported that mortality, morbidity and evidence of stress were higher for pullets that were fed diets containing 2 or 4 μg ochratoxin.g^{-1} than for birds fed a control diet. In addition, lower body-weight gains during the first 14 days post-hatching were observed for the progeny of hens fed the ochratoxin-containing diets, but between 14 and 28 days these progeny had weight gains similar to those from dams fed the control diet. Prior et al. (1980) found that differences among body-weight gains related to dietary ochratoxin A levels disappeared after 6 to 7 weeks when broiler chickens were fed diets containing 0.5 or 1.0 μg toxin.g^{-1}.

In the case of other mycotoxins, the effects on livestock and poultry do not necessarily occur immediately and may persist long after they start to appear. For example, Wyatt et al. (1973) observed that the growth of broiler chickens was not retarded by T-2 toxin until two weeks after starting to feed the toxin-containing diets.

7. Interactions between mycotoxins. This is probably one of the most important parameters. Frequently more than one mycotoxin occurs in naturally-contaminated feedstuffs. Published results indicate that DON or DON-derivatives occur with zearalenone in naturally-contaminated corn (Young et al. 1981, 1982; Etienne and Jemmali 1982) and white winter wheat (Friend et al. 1982), monoacetoxyscirpenol with zearalenone in

corn artificially inoculated with F. roseum (Speers et al. 1977) and citrinin with ochratoxin in naturally-contaminated barley (Madsen et al. 1982a). Other mycotoxins may also be present, probably below the limit of sensitivity of present analytical methods; Allen et al. (1983) found (in $\mu g.g^{-1}$) zearalenone (14 000), scirpentriol (42), monoacetoxyscirpenol (41), diacetoxyscirpenol (DAS) (64) and 4-acetoxyscirpenol (11) in parboiled rice inoculated with F. roseum "Gibbosum". They also detected T-2 toxin (1109), neosolaniol (220), HT-2 toxin (74), acetyl T-2 toxin (106), 8-acetylneosolaniol (47), TMR-1 toxin (13), TMR-2 toxin (3.2), NI toxin (1.3), T-2 tetraol (1.3) and deacetyl HT-2 toxin (0.5) when parboiled rice was inoculated with F. tricinctum.

There is evidence that the performance of poultry and swine may, on occasion, be influenced by interactions between mycotoxins present in the diet. Hoerr et al. (1981) concluded that when broiler chickens were given either a single dose or 14-day multiple oral doses, T-2 toxin and DAS had additive lethal toxicity when dosed in combination. Huff and Doerr (1981) found that the separate administration of aflatoxin and ochratoxin A resulted in a 12% decrease in body weight of broiler chickens at 3 weeks of age, but when administered in combination, the toxins produced a 40% decrease in body weights. This indicates that a synergistic interaction occurred between these mycotoxins. Campbell et al. (1983) also reported that aflatoxin and ochratoxin A had a synergistic effect on the body weight of broilers. With swine, however, Madsen et al. (1982a) reported that purified ochratoxin A and citrinin, alone or in combination, had no significant influence on daily feed and water intake, body-weight gain and feed efficiency.

5.2.2 EFFECTS OF MYCOTOXIN ON LIVESTOCK AND POULTRY PRODUCTION

Several reviews have recently been published summarizing the effects of mycotoxins on the productive performance of cattle (Carlton and Tuite 1980), cattle, swine and poultry (Pier et al. 1976, 1980) and poultry (Wyatt 1979; Smith 1981, 1982a). Productive performance is a function of feed intake and efficiency, growth (body-weight gain) of young animals, mortality, milk (cows) and egg (hens) production, egg and shell quality (hens), and reproductive efficiency. The latter is dependent, in male animals and birds, on libido and semen quality and quantity and, in female animals, on the number of matings required for conception, the number of animals born alive, the birth weight of live born animals, the growth and mortality of the progeny during the first two to four weeks post-partum and on milk production. In hens, reproductive efficiency is dependent on egg production, fertility, hatchability of fertile eggs, body weight of chicks and poults (turkeys) at hatching, and growth and mortality of progeny during the first several weeks post-hatching. These are the major animal-related variables that directly influence the economic returns of livestock and poultry producers.

One of the first detectable biological effects often observed when young livestock and poultry ingest several of the mycctoxins, alone or in combination, is a decrease in feed intake with a concomitant reduction in body-weight gains (growth). Feed efficiency, expressed in terms of feed/gain ratio or feed per unit of egg weight produced, may or may not be affected. Published results indicate that there is an inverse relationship between body-weight gains or feed intake of various animals and dietary levels of various mycotoxins (Table 5-1).

The levels of mycotoxins ingested in cases of chronic mycotoxicosis are generally lower by several times than those that produce acute mycotoxicosis in livestock and poultry. As a result, increases in mortality directly related to chronic mycotoxicosis may not appear. However, the suppression of biological mechanisms of resistance and of immunological response by dietary mycotoxins may increase the occurrence of secondary infections which eventually cause death and thereby increase mortality (Pier et al. 1980). Complement activity has been postulated to be the most sensitive aspect of the immune system of broiler chickens influenced by a combination of aflatoxin and ochratoxin A (Campbell et al. 1983).

Hsu et al. (1972) noted lower reproductive efficiencies because of an increase in the frequency of abortions of lactating cows that ingested T-2 toxin-contaminated feed during gestation. Weaver et al. (1978c) observed reduced fertility, litter size and body weight at birth of piglets from mature sows (3 years old) fed a diet containing T-2 toxin from before estrus to farrowing. Berger et al. (1981) reported reduced libido and mating behavior of boars and Chang et al. (1979), Etienne and Jemmali (1982) and Long et al. (1983) noted a reduction in fertility, litter size, body weight at birth and early post-partum liveability of piglets, and an increase in the occurrence of constant estrus and pseudo-pregnancy of both young and mature sows given diets that contain zearalenone. In the case of Leghorn hens, ochratoxin has been associated with delayed sexual maturity (Choudhury et al. 1971) and ochratoxin and T-2 toxin have been linked with reduced egg production (Choudhury et al. 1971; Chi et al. 1977; Speers et al. 1977; Prior and Sisodia 1978; Hamilton et al. 1982), hatchability of fertile eggs (Choudhury et al. 1971; Chi et al. 1977; Speers et al. 1977) and early chick growth (Choudhury et al. 1971). Prior and Sisodia (1978), however, found that diets containing purified ochratoxin A had no effect on fertility and hatchability of eggs from hens consuming these diets. Ingestion of diets containing up to 800 µg zearalenone.g^{-1} did not influence egg production, fertility of hatchability of fertile eggs from pullets and hens (Speers et al. 1971; Marks and Bacon 1976; Allen et al. 1981). Furthermore, semen fertility of roosters fed diets containing up to 800 µg zearalenone.g^{-1} and hatchability of fertile eggs from hens inseminated with semen from these roosters were not affected by this estrogenic mycotoxin (Allen et al. 1981). Semen quantity and quality of mature boars were also found not to be influenced by dietary levels of zearalenone up to 200 µg.g^{-1} (Ruhr et al. 1983).

Table 5-1. Data supporting inverse relationship between body-weight gains or feed intake and dietary levels of mycotoxins.

Animal studied	Mycotoxin with which effect was observed	References
Calves, cows	T-2 toxin	Hsu et al. (1972) Pier et al. (1976) Petrie et al. (1977)
	zearalenone	Noller et al. (1979)
Swine	DON	Forsyth et al. (1977) Schuh et al. (1982) Koch and Pollmann (1982) Pollmann and Koch (1982) Friend et al. (1982, 1983a)
	ochratoxin	Madsen et al. (1982a)
	zearalenone	Young et al. (1981, 1982, 1983b)
Chickens, turkeys	ochratoxin	Peckham et al. (1971) Huff et al. (1974) Prior et al. (1976, 1978) Hamilton et al. (1982)
	T-2 toxin	Wyatt et al. (1973) Speers et al. (1977) Chi et al. (1977) Hoerr et al. (1982)
	zearalenone	Speers et al. (1971) Adams and Tuite (1976)

There is some evidence that egg and shell weight and shell thickness are reduced by increased levels of dietary DON (Hamilton et al. 1981), egg shell quality is decreased by ochratoxin (Hamilton et al. 1982) and shell thickness is diminished by T-2 toxin (Chi et al. 1977). A relationship has been observed between dietary DON level and liver lipid levels of Leghorn hens (Farnworth et al. 1983).

5.3 PREVENTION AND TREATMENT OF MYCOTOXICOSES (T.K. Smith)

5.3.1 INTRODUCTION

In some cases, a simple and effective method for the prevention and treatment of mycotoxicoses is dietary manipulation. This has been demonstrated for aflatoxicosis of various species (Patterson 1983). The adequacy of dietary protein can markedly influence the metabolism of aflatoxin (Adekunle et al. 1977). Dietary protein supplements beyond normal requirements, therefore, can be used to treat acute aflatoxicosis in swine (Sisk and Carlton 1972), poultry (Smith et al. 1971) and many other species (Smith 1982b). The benefits of supplementary vitamins and lipotropes are also under investigation (Newberne et al. 1968; Hamilton et al. 1974).

Non-nutritive dietary components can also influence aflatoxicosis. Frape et al. (1981) reported that fibre sources promote fecal excretion of aflatoxin while Masimango et al. (1979) indicated that clays can bind aflatoxin in vitro.

The above observations have prompted the testing of numerous dietary components for the ability to overcome Fusarium mycotoxicoses.

5.3.2 ZEARALENONE

The effect of diet on zearalenone toxicosis has been tested in rats and swine (Smith 1980a). Sources of natural fiber, such as alfalfa meal and oat hulls, were particularly effective, as were anion-exchange resins. Protein supplements had a slightly beneficial effect. James and Smith (1982a) showed that alfalfa could reduce not only tissue residues of zearalenone and zearalenols, but also the uterotropic effect of these compounds. Studies with ^{3}H zearalenone, however, failed to show any marked increase in fecal excretion of zearalenone with alfalfa feeding (Smith 1980b). Subsequent experiments using the more specific high pressure liquid chromatography (HPLC) technique of James et al. (1982) have confirmed the last observation (Smith 1982c). It is unlikely that alfalfa influences the metabolism of zearalenone, since Stangroom (1983) failed to find any significant effect of dietary alfalfa or its fractionated components on the activity of hepatic hydroxysteroid dehydrogenase, an enzyme believed to metabolize zearalenone to zearalenols. These studies also showed that the fiber

fraction of alfalfa was most effective in overcoming zearalenone toxicosis. It is likely, therefore, that beneficial effects of dietary alfalfa are due to small reductions in the absorption of zearalenone from the intestinal tract. The feeding of dehydrated alfalfa remains the most effective and practical method yet devised to prevent or treat zearalenone toxicosis through dietary manipulation.

5.3.3 TRICHOTHECENES

Studies of the effect of diet on trichothecene toxicoses have mainly used T-2 toxin (Smith and Carson 1984), probably because our understanding of the metabolism of T-2 toxin is better than it is for other trichothecenes, and because T-2 toxin is readily available commercially in purified form. Hayes and Schiefer (1980) found that dietary protein supplements partially alleviated T-2 toxicosis in mice while Davis and Schiefer (1982) made similar observations with respect to the yellow mealworm.

The potential benefits of high fiber diets have also been investigated (Carson and Smith 1983a). Purified cellulose, hemicellulose, pectin and lignin had little effect on feed refusal in rats caused by T-2 toxin. Alfalfa meal, however, overcame feed refusal and reduced tissue residues while reducing intestinal transit time for the toxin. Similar benefits were noted when bentonite was included in the diet (Carson 1982; Carson and Smith 1983b). Diets high in animal or vegetable fat have also been reported to overcome T-2 toxin-induced feed refusal in rats, although the mechanism by which this occurs is not known (Smith et al. 1980).

More recently, it was reported that feed refusal due to T-2 toxin could be overcome by the feeding of spent canola oil bleaching clays, a byproduct of the canola seed crushing industry (Smith 1983). This protective effect is due to a combination of the residual oil (about 27% of the product) and the clay.

5.3.4 CONCLUSIONS

Dietary treatment shows promise as a method for preventing and treating Fusarium mycotoxicoses. Combinations of toxins are common in nature, and in this regard, high fiber diets containing alfalfa meal are particularly useful as they overcome the adverse effects of both zearalenone and T-2 toxicosis. It is likely that such treatments will also prove effective against other trichothecenes because of their similar chemical structures and polarity. Further testing will be necessary as purified supplies of trichothecenes become available in quantity. The concept of using waste byproducts from the oilseed crushing industry to overcome mycotoxicoses in livestock should be explored further as it offers potential solutions to the problems of impaired animal performance and industrial waste disposal.

Progress must also be made in identifying residues of trichothecene metabolism in animals and determining the relative toxicity of these compounds. This will be of importance in determining the safety of food products of animal origin when such foods contain mycotoxin residues. It is likely that dietary treatments that overcome trichothecene-induced feed refusal will also reduce tissue residues of toxic metabolites. This has been shown in studies with ^{3}H T-2 toxin, although the chemical structure of the majority of the ^{3}H metabolites was not determined.

5.4 REGULATION OF MYCOTOXINS IN FEEDS (J.F. Standish)

Livestock feeds produced or sold in Canada are regulated under the authority of the Canada Feeds Act and Regulations. Although few Regulations pertaining specifically to mycotoxins are included, both the Act and the Regulations stipulate that it is an offence to manufacture, import or sell any feed which may be harmful to livestock or to the environment. They further prohibit the manufacture, importation or sale of any feed which may result in the production of an adulterated food product under the Food and Drugs Act (Health and Welfare Canada 1981).

The Canada Grain Grading Guide (Canadian Grain Commission 1982) specifies the maximum limits of ergot allowed in various grades of wheat, rye and other grains. For example, the maximum percentage of ergot allowed in Nos. 3 and 4 grade Canada western rye after removal of dockage is 0.33%. The considerable variations in alkaloid content and composition of ergot sclerotia, and in the responses of different animal species to ergot, make it difficult to assess the safety of particular animal feed samples. However, it has been generally recommended that feeds containing more than 0.1% ergot by weight not be given to animals (Young 1979, 1981a).

The Feeds Regulations (Government of Canada 1983) approved by the Governor-in-Council on 14 July 1983, include a Regulation prohibiting the manufacture or use of livestock feeds containing aflatoxin at levels in excess of 20 $\mu g.kg^{-1}$. Agriculture Canada laboratories carry out a routine monitoring program for specific mycotoxins in livestock as well as mycotoxin tests on suspect feeds when livestock production problems are reported.

Because of the generality of the regulations pertaining to feed safety, the Feed and Fertilizer Division, Agriculture Canada, has in the past indicated certain policies relating to mycotoxin contamination in livestock feeds. When DON was identified in Ontario white winter wheat in 1980, the Feed and Fertilizer Division, in consultation with research scientists, issued a memorandum to the feed trade in eastern Canada on their concerns about this toxin and indicated what was considered to be safe usage levels for this crop. In 1980, a recommendation to restrict maximum use to 25% Ontario white winter wheat

in livestock rations was made. For Québec wheat, where DON concentrations were higher, the maximum recommended usage level for wheat in livestock and poultry rations was set at 10%. In 1981, the DON concentration in Ontario was found to be considerably lower and no recommendations were made concerning maximum usage levels for that crop. The 1981 Québec wheat crop, however, was found to contain higher average concentrations of DON, with identification of some extremely high level samples. On the basis of these results, the 10% maximum inclusion rate suggestion was reiterated to feed manufacturers.

An extensive research effort initiated by Agriculture Canada, Health and Welfare Canada, Canadian university researchers and scientists abroad (Hamilton et al. 1981, 1983; Scott et al. 1981; Young et al. 1981, 1982; Friend et al. 1982, 1983a; Moran et al. 1982; Schuh et al. 1982; Trenholm et al. 1982, 1983; Smith 1983) indicated, by the time the 1982 crops were harvested in eastern Canada, that livestock could tolerate somewhat higher concentrations of DON than originally thought, and no recommendations concerning wheat usage were made.

In June 1983 Agriculture Canada organized a meeting of scientists carrying out studies on the effects of feeding DON-contaminated diets to livestock and poultry. After reviewing relevant experimental evidence on the safety of DON in livestock and poultry feeds and feedstuffs and the general levels of DON found in grain samples taken as part of recent Canadian surveys, the researchers concluded that, with the possible exception of swine, the feeding of eastern Canadian wheat to livestock should not result in apparent harmful production effects. On the basis of this conclusion, it is doubtful that further Departmental advice will be necessary relating to the use of DON-contaminated grain in livestock rations.

6.0 POTENTIAL HUMAN HEALTH HAZARDS AND REGULATORY ASPECTS

(T. Kuiper-Goodman)

6.1 INTRODUCTION

Ill effects can occur in humans because of exposure to mycotoxins. Our main concern is the dietary intake by humans of mycotoxins or their metabolites either from food crops (direct intake) or from animal tissues or products of animals that have ingested mycotoxin-contaminated feed (indirect intake). At present no known human toxicoses of Canadians can be attributed to the dietary intake of mycotoxins. This is mainly due to the low levels generally present, as well as our marketing distribution system, in which the produce of many farms is mixed or blended, distributed and consumed by many people. However, human mycotoxicoses due to ingestion of mycotoxin-contaminated food are either well-documented or presumed elsewhere. One must also keep in mind that slight signs or symptoms of mycotoxicoses may be overlooked or attributed to other causes.

While most of the documented or presumed human mycotoxicoses are due to ingestion of high levels of mycotoxins in contaminated food for a relatively short period of time (up to several months), an association between a high incidence of human liver cancer and a high incidence of relatively low levels of aflatoxins in dietary staple foods has been found in several countries (Section 6.3.1). Our health hazard evaluations and regulatory efforts are directed towards ensuring that mycotoxins in foods are kept at levels well below the values that may constitute potential health hazards.

Human exposure to mycotoxins can also occur through the dermal route or through inhalation, as during grain handling on the farm, in grain elevators, and in food and feed processing. Little is known about human mycotoxicoses due to these occupational exposures, and there are no regulations in Canada or elsewhere with respect to allowable levels of mycotoxins in airborne dust (see Section 1.4). In general the occupational routes of exposure are considered to be of much less importance than the dietary route, and more emphasis will be placed on the latter.

Health concerns related to the dietary intake of mycotoxins depend on the levels of mycotoxins in the food, as consumed, on the amount of food consumed, on the body weight and physiological state of the individual and on the toxicity of the compound. Other dietary factors may increase or decrease the toxicity (cf Section 5.3). Government regulations with respect to mycotoxins reflect our health concern.

6.2 MYCOTOXIN LEVELS IN FOOD

6.2.1 DIRECT INTAKE

The occurrence of mycotoxins in Canadian food and feed crops was discussed in Section 2.0. Mycotoxin levels can be reduced by specific (chemical) decontamination practices (see Section 8); these decontamination practices would have to be reviewed for safety and at present none is being used for food in Canada. Normal processing of raw food either at the manufacturing level (drying, heating, sorting, milling) or at home may reduce the level of mycotoxins in the food, as consumed, depending on their heat stability and solubility (Scott 1984b). Processing may also cause isomerization and other breakdown products, the safety of which needs to be considered. In our safety assessments, information on processing and decomposition of mycotoxins, when available, is considered.

6.2.2 INDIRECT INTAKE

To assess the indirect intake of mycotoxins through the consumption of animal tissues and produce from animals that were fed mycotoxin-contaminated feed, feed-food "carry over" studies are required to determine the nature of residues in animal products.

In such studies the mycotoxin of concern is fed to different types of livestock, at a specific level in the feed for a specific time period, and the resulting level of that mycotoxin or its metabolites in the animal tissues or produce likely to be consumed (muscle, liver, milk, eggs) is measured. Such studies have been conducted for aflatoxin and ochratoxin, and are under way for some of the trichothecenes. It is obvious that if the "carry over" is slight, analytical detection may be difficult and require the development of new methodology, including the use of tracer techniques where feasible.

Thus, when initially confronted with a problem of a food or feed crop containing a mycotoxin, one will have the make conservative estimates with regard to the level of mycotoxins in the food as consumed until more precise data are available.

6.2.3 ESTIMATES OF FOOD INTAKE

Estimates for the intake of various foods differ from country to country and within a country, and with age, sex, body weight and social, economic and ethnic preferences. Estimates of food intake are made using several surveys.

In Canada use is made of "Canada disappearance figures" (Statistics Canada) which provide a mean per capita intake, but which do

not take into account non-eaters or removal from food use to alternate use. A dietary recall survey covering a 24-hour or 1-week period has been made (Health & Welfare Canada, 1976). In this survey, the amount consumed of a variety of foods (as consumed) was tabulated by sex and age groups. Eaters and non-eaters could be distinguished. Other North American surveys and specific surveys geared towards special segments of the population are also available (e.g. infants, preschoolers (Yeung et al. 1979; Leung et al. 1984)).

None of these surveys is complete for each food item. Combining the estimates of food intake with the estimates of level of mycotoxins found in food/feed crops or foods derived from these gives an estimate of mycotoxin intake. Both the mean intake of eaters and the higher estimate based on the 90th percentile of eaters are examined.

6.2.4 TOXICOLOGY

In general, laboratory animals are used to predict if adverse effects are likely to occur in man. The type of toxicological data required to assess possible toxic effects in man varies with each compound. Exposure and available biochemical and toxicological data are important factors to be considered. Data are required with respect to acute toxicity, 30-90 day toxicity, metabolism, reproduction (2-generation, 4 litters), teratogenicity, mutagenicity, and carcinogenicity. Usually studies are conducted in a rodent (preferably rat) and a non-rodent species, and if possible the species used should metabolize the compound in a manner similar to that of man. For livestock studies, oral administration is the preferred route. In general, these studies are conducted in government laboratories or universities.

If epidemiological data are available for man that indicate an association of disease and exposure to toxin (e.g. liver cancer and aflatoxin exposure), such data may also be used, bearing in mind the influence of other factors such as nutritional status, endemic infections, intake of other toxic substances, etc. Known adverse effects in livestock are also taken into account in the assessment.

When we relate dietary exposure data and toxicity data we usually express both on a body weight basis. Infants and children may be more susceptible because their basal metabolic rate is greater and they may not possess the biochemical mechanisms to detoxify toxic substances. In children, organs such as the brain continue development for many years after birth, and this makes them more susceptible to substances affecting the CNS. Therefore we sometimes deal separately with children.

6.2.5 REGULATION

Other than for aflatoxins, few regulations exist in Canada or elsewhere with respect to mycotoxins in food (Schuller et al. 1982). Most countries have adopted the policy that unless it is necessary to limit the levels of mycotoxins in certain foodstuffs because of a known mycotoxin problem, no regulations or guidelines are needed. Thus, in general, no guidelines are in existence if the levels of mycotoxins in the food have not been identified as a reason for health concern.

For mycotoxins that are a health concern, a tolerable daily intake can be estimated, when the toxicological data base is sufficient, by extrapolating the relevant toxicological data to man using appropriate safety factors or other means of extrapolation and evaluation. A comparison of the estimated daily intake (6.2.3) of a mycotoxin and the tolerable daily intake or tentative tolerable daily intake gives us an indication of the degree of concern. When the estimated daily intake of a mycotoxin exceeds its tolerable daily intake, steps must be taken to reduce the intake of that mycotoxin, namely by completely removing the offending food or by setting a tolerance or interim guideline levels to limit the amount or level of the mycotoxin that can be tolerated either in the raw food crop or in a finished food. Analytical methods have to be available to ensure compliance with a tolerance (see Section 7.0).

Similarly, to limit the indirect intake of mycotoxins, tolerances may be set on the maximum levels of mycotoxins that can be allowed in feed (Section 5.4). In other countries these tolerances may be restricted to certain types of livestock (e.g. dairy vs beef cattle; layer vs broiler chickens). In the United States, where there has been a problem with aflatoxin contaminated feed, it was found difficult to ensure adherence to the restrictions for different livestock species.

6.3 MYCOTOXINS IN FOOD WITH POSSIBLE HEALTH IMPLICATIONS

The individual mycotoxins that have been considered in Canada for possible health implications in the Canadian food situation are aflatoxin, zearalenone, ochratoxin, patulin, ergot alkaloids and deoxynivalenol (DON, vomitoxin). Two of these, aflatoxin and DON, are discussed in the following sections. No Canadian regulations are in effect for the others.

6.3.1 AFLATOXINS

Concern with regard to aflatoxins started in the 1960's, after some 100,000 turkey poults in Great Britain died because of aflatoxin exposure from their feed (see Heathcote and Hibbert 1978; Patterson 1983). When it became evident that aflatoxin exposure caused cancer in

many species tested, most countries, including Canada, set tolerances for aflatoxin levels in food or feed in order to limit exposure to this group of mycotoxins (Schuller et al. 1982).

6.3.1.1 Toxicology

Of the aflatoxins present in food, aflatoxin B_1 and the metabolite M_1 are of most concern. Although both are carcinogenic, aflatoxin B_1 is the more potent. Very low dietary levels of aflatoxin B_1 (1 $\mu g.kg^{-1}$) have caused liver cancer in the Fischer rat (Wogan 1973); a no-effect level (NOEL) has not yet been established in this strain of rat.

Although aflatoxin B_1 is carcinogenic in all species tested, there are significant differences with regard to sensitivity. Species more susceptible to the acute effects of aflatoxins tend to be less sensitive to tumor induction and vice versa. These differences have been explained in part by species differences in metabolism (Hsieh et al. 1977; Shank 1977). Aflatoxin metabolism in the liver, which includes detoxification as well as activation, is influenced by age, hormonal status and composition of diet (see Patterson 1983). Based on information on the metabolism of aflatoxins in man it has been postulated that man may be less sensitive to the long-term effects of aflatoxin metabolites than the Fischer rat. Aflatoxin also appears to be a less potent mutagen in man than in rats, based on the Ames assay (Hsieh et al. 1977). It is, however, difficult to ensure comparability in such studies. These species differences are taken into account when extrapolating the significance of animal data on tumor induction to man.

6.3.1.2 Epidemiology

There have been several published reports of acute aflatoxicosis in man, many with a fatal outcome (Patterson 1983). The largest documented outbreak, due to consumption of aflatoxin contaminated corn, occurred in India in 1975 (about 400 cases; 25% fatal). It was estimated that up to 6 mg aflatoxin B_1 per person was consumed (Krishnamachari et al. 1975). Follow-up studies are being conducted in affected patients (Bhat and Krishnamachari 1977). In other outbreaks of acute human aflatoxicosis that involved fatalities, levels of 200-300 $\mu g.kg^{-1}$ aflatoxin B_1 were found in part of the diet. The symptoms of acute aflatoxicosis resemble those seen in Reye's syndrome; aflatoxin has been suggested as one of the causes of that disease. Aflatoxin B_1, M_1 or other aflatoxin metabolites have also been detected in the liver tissue and urine of a variety of human subjects (Patterson 1983).

Epidemiological studies have shown an association between dietary aflatoxin exposure and the high incidence of primary liver

cancer in a number of African and South East Asian countries (see Section 1.4). The role of other factors, e.g. hepatitis B virus, that may influence this association has not been fully investigated.

In Canada, exposure to aflatoxin is one of the lowest in the world and the incidence of liver cancer is also one of the lowest. Extrapolating from the epidemiological data obtained in other countries gives a crude estimated increase in primary liver cancer rate of $0.16/10^5$ person for each ng aflatoxin B_1.kg b.w.$^{-1}$.d^{-1} consumed. It has been postulated that dietary and other factors may protect North Americans to some extent (Stoloff 1983b).

6.3.1.3 Foods of Concern

As shown in Section 2.2.4 the major foods of concern in Canada are peanuts and peanut butter because these foods are consumed widely by children. In Canada there appear to be no other significant sources of aflatoxin, except for occasional exposure to other types of contaminated nuts, especially brazil nuts (cf. 6.3.1.4). A recent survey of Canadian feeds (Section 2.1) has shown that aflatoxins in feed is not a problem in Canada; we do not have to be concerned about the carry-over of aflatoxin M_1 into milk.

6.3.1.4 Effects of processing

Peanuts are subjected to electronic sorting in processing to peanut butter by major industries, with the aim of removing poor quality nuts and thus reducing aflatoxin levels in the final produce as monitored by *in house* batch sampling. The roasting of peanuts also reduces their aflatoxin level by 50% or more (see review, Scott 1984b).

Brazil nuts and pistachio nuts have been susceptible to aflatoxin contamination; levels as high as several parts per million have been found in individual nuts. When they are sold in the shell, the responsibility for handsorting and removing poor brazil nuts is shifted to the consumer. Canadians have been warned against mold on nuts by the HPB and told to reject such nuts (Health and Welfare Canada 1982).

6.3.1.5 Intake of Aflatoxins

Based on an estimated level of 1 ng aflatoxin $B_1.g^{-1}$ of peanut butter (from HPB monitoring surveys), the mean estimated daily intake of aflatoxin B_1 from peanut butter is 0.6 ng.kg b.w.$^{-1}$ (for 5-9-year-old children) and the 90th percentile intake for this age group is 1.6 ng.kg b.w.$^{-1}$ (Kuiper-Goodman *et al.* 1979). As pointed out before, other sources of food do not significantly alter this figure. The intake for other age groups on a body-weight basis is lower.

6.3.1.6 Tolerances in Effect in Canada

No tolerable daily intake has been established. Instead, a tolerance of 15 $\mu g.kg^{-1}$ (total aflatoxin) for nuts and nut products is in effect (Health and Welfare Canada 1981). The tolerance for aflatoxins was set with respect to analytical methods available in 1976, and to levels which were technically achievable at that time. For the purpose of this regulation, the aflatoxin content of a nut or nut product shall be determined excluding any shell.

6.3.2 DEOXYNIVALENOL (DON, VOMITOXIN)

Concern with regard to DON started in 1980 when pink wheat kernels and elevated levels of DON were found in Ontario winter wheat (soft wheat) and Québec spring wheat (hard wheat) (Scott et al. 1981; Trenholm et al. 1983; Scott 1984c). DON has also been found in some corn and barley.

6.3.2.1 Toxicology

Deoxynivalenol belongs to a group of more than 50 naturally occurring 12,13-epoxytrichothecenes (Ueno 1983). The trichothecene mycotoxins, as a whole, possess similar chemical and biological features, characterized by antibiotic, antifungal and cytostatic activities. Many were isolated as causative toxicants in etiological studies of naturally occurring intoxications associated with moldy foods and feeds. The acute symptoms of trichothecene poisoning are characterized by: skin irritation, feed refusal, vomiting, diarrhea, hemorrhages, neural disturbance, abortion and death. Deoxynivalenol belongs to the B group of trichothecenes. In 1980, there was very little relevant toxicological information available on DON. As pure DON became available, this situation has improved. Biochemically, trichothecenes, including DON, are inhibitors of protein and DNA synthesis. So far, only negative results have been obtained in mutagenicity tests (Rogers and Héroux-Metcalf 1983). Short-term effects of oral intake of DON by rodents (gavage, diet), as well as effects on reproduction, including teratogenic potential, were assessed at the Health Protection Branch (Khera et al. 1982, 1984). These studies have shown a preliminary no effect level (NOEL) of 0.75 $mg.kg\ b.w.^{-1}$. At higher dosage levels, reproductive, embryotoxic, and possible teratogenic effects were noted. Deoxynivalenol also affected food intake, body weight and the immune system. Further studies in rodents and non-human primates will focus on the elucidation of the long term toxicity, oncogenicity, immunotoxicity, pharmacokinetics, metabolism, and mechanism of action. Feeding studies on livestock and poultry species are discussed in Section 5.1.2-3.

6.3.2.2 Foods of Concern

In Canada, DON has been found mainly in wheat grown in the eastern provinces and, in particular, in soft wheat, which is chiefly used in the production of nonstaple foods such as cakes, cookies, biscuits, and cereals. The major Canadian wheat crop, Western hard wheat, which is used for the most part in staple foods (bread), is not affected (Scott 1984c). Products made from kernel corn (e.g. corn breakfast cereal, popcorn) are also of concern.

6.3.2.3 Effects of Processing

Studies by Agriculture Canada and industry, have shown that, in general, DON levels decrease by about 40% during industrial processing from uncleaned soft wheat to the flour portion of the finished food product, except for yeast doughnuts which appeared to contain increased amounts of DON (Young et al. 1984a) (see Section 8.1).

6.3.2.4 Intake of Deoxynivalenol

Estimates of intake by males, 12-19 years old, were made for various food categories (bread, cakes, cookies, cereals, etc.) and the intake of DON was estimated based on its level in the various food categories. As information on processing becomes available the intake estimates for DON are being adjusted.

6.3.2.5 Tolerances in Effect in Canada

Based on the toxicity information on DON available early in 1983 a tentative tolerable daily intake of 3.0 $\mu g.kg\ b.w.^{-1}$ for adults and 1.5 $\mu g.kg\ b.w.^{-1}$ for infants was established.

Preliminary guideline levels of DON were therefore adjusted upwards in 1982 to 2.0 $\mu g.g^{-1}$ from 0.6 $\mu g.g^{-1}$ in uncleaned soft wheat used for non-staple foods including bran, except for soft wheat destined for infant food where the guideline level is 1.0 $\mu g.g^{-1}$. As a result of processing, levels of 1.2 $\mu g.g^{-1}$ and 0.6 $\mu g.g^{-1}$ DON would be expected in the flour portion of finished foods and infant foods respectively.

For the 1982 and 1983 eastern Canadian winter wheat crops, the HPB allowed blending of uncleaned soft wheat in order to bring such wheat into compliance with the guideline levels. For domestic products, the HPB restricted its regulatory action to the final blend of uncleaned soft wheat prior to milling. Imported products, on the other hand, were subject to regulatory action based on the level of DON found in the flour portion of the finished product.

With regard to (hard) wheat grown in eastern Canada for use in staple foods, the level of DON in the final blend of harvested cleaned wheat should not exceed 0.5 $\mu g.g^{-1}$.

7.0 METHODS OF ANALYSIS

(P.M. Scott)

7.1 INTRODUCTION

The detection and measurement of mycotoxins constitute an essential aspect of research and regulation in this area. We need good methodology to carry out surveys of mycotoxins in foods and feeds and to determine human and animal exposure; to exercise control at commodity, manufacturing and retail levels; to assess stability of mycotoxins during storage, processing and decontamination; to conduct toxicological studies with naturally-contaminated materials; and to carry out other research such as epidemiology, metabolism studies, and microbiological production.

Since 1963, considerable effort has been devoted to developing methods for the analysis of mycotoxins. In 1979, the U.S. Food and Drug Administration's computerized data base contained over 1300 methods, 38% of which concerned aflatoxins (Pohland et al. 1979). It must be realized, however, that the reliability of any assay of an agricultural community for mycotoxins is largely dependent on sampling. A single peanut may contain up to 0.1% aflatoxins and only a few kernels per thousand may be contaminated. Thus if a given lot of peanuts contained 20 $\mu g.kg^{-1}$ of aflatoxins distributed heterogeneously, it has been determined that sampling contributes at least two-thirds of the total variability of the aflatoxin content, subsampling of the ground sample about 20%, and the actual analysis the remaining 13% (Horwitz and Albert 1982). Similar studies have been carried out with aflatoxin in shelled corn and it was found that in this case the sampling variation was about the same as the analytical variation (Whitaker et al. 1979). Protocols for surveys, sampling, post-collection handling, and analysis of grain samples relative to mycotoxin problems have been recommended by Davis et al. (1980). These warrant consideration for application to Canadian problems, such as DON in corn and wheat, where sampling variability studies are urgently needed. Problems of obtaining samples of large-animal tissue are of a different kind: edge samples of aflatoxin-contaminated beef liver averaged 44% lower aflatoxin concentrations than the rest of the lobe samples, indicating that wedge cuts from a liver would be representative (Stubblefield et al. 1981).

7.2 TYPES OF METHODS

Analytical methods may be broadly divided into quantitative or semiquantitative assays and rapid screening tests; into methods for individual mycotoxins and multimycotoxin methods; into physicochemical determinations and bioassays; and into presumptive and confirmatory techniques. Methodology for mycotoxins in general has been reviewed by

Stoloff (1972), Pohland et al. (1979) and Egan et al. (1982) and there are recent reviews on methods for aflatoxins (Nesheim 1979), ochratoxins (Harwig et al. 1983), zearalenone (Bennett and Shotwell 1979), and trichothecenes (Scott 1982a), in particular. Sample cleanup is an important, time-consuming step and usually involves extraction with solvent, liquid-liquid partition and/or column chromatography, and, in some methods, gel permeation chromatography, preparative thin layer chromatography (TLC), or preparative high performance liquid chromatography (HPLC). The detection-quantitation step has classically been TLC (Scott 1980, 1982b) using visual or densitometric determination. This simple, inexpensive technique should not be abandoned in favor of instrumental measurement by HPLC (Scott 1981a) or gas-liquid chromatography (GLC) (Romer 1984) when a choice not dictated by sensitivity or specificity requirements is possible. TLC is still the most commonly used technique (Pohland et al. 1979) and is especially useful for aflatoxin analyses, screening purposes, and multimycotoxin analysis. Furthermore, in a recent collaborative study on aflatoxins in cottonseed products (Pons et al. 1980), within-laboratory error (repeatability) was essentially the same with both TLC and HPLC, although use of the latter did reduce the between-laboratory error component. High performance radial chromatography is a more recent technique that greatly improves the resolving power of high performance TLC precoated plates; it has been applied to determination of aflatoxins in peanut and cottonseed products by Vitek et al. (1979). For some mycotoxin analyses, e.g. ergot alkaloids in flour (Scott and Lawrence 1980, 1982), HPLC is clearly preferable to TLC for separation and sensitivity, while for others, e.g. vomitoxin and trichothecenes in general, GLC with electron capture detection or mass spectrometric single ion monitoring is superior (Scott 1982a). Other quantitation techniques include immunoassay, notably enzyme-linked immunosorbent assay (ELISA) (El-Nakib et al. 1981; Pestka et al. 1981), fluorimetry, and polarography (Pohland et al. 1979). Recently, the use of tandem mass spectrometry (MS/MS) has been explored for the analysis of aflatoxin B_1, T-2 toxin and vomitoxin (Davidson et al. 1982).

Quantitative methods are essential for enforcement purposes. Thus, for example, it is required under the Canada Food and Drugs Act and Regulations that nuts and nut products (excluding shell) contain no more than 15 $\mu g.kg^{-1}$ of aflatoxin. However, for the screening of commodities for aflatoxins in field situations by inexperienced personnel, simple and economical minicolumn methods are widely used, particularly in the United States (Romer et al. 1979; Holaday 1981; Shotwell and Holaday 1981). Minicolumn methods have also been developed for ochratoxin A (Hald and Krogh 1975; Holaday 1976) and for zearalenone (Holaday 1980). The ultimate rapid test involves the detection of fluorescing particles in corn samples: Bright Green Yellow (BGY) fluorescence. This test indicates the presence of aflatoxin (Dickens and Whitaker 1981) although it is based on detection of another *Aspergillus flavus* metabolite. False positives and false negatives with the BGY fluorescence test can range up to 31% and 4% of samples, respectively (Lillehoj 1979).

Methods for aflatoxins B_1, B_2, G_1, G_2 and M_1, ochratoxins, patulin, sterigmatocystin and zearalenone that have been validated in interlaboratory studies are described in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) (Stoloff 1980). This compilation is regularly updated and for a continuing review of mycotoxin methodology (not limited to official methods) the annual reports of the AOAC General Referee for Mycotoxins should be consulted (Stoloff 1982a, 1983c; Scott 1984a). At present there are no official multimycotoxin methods. These are becoming increasingly sophisticated in their judicious use of TLC systems, so that up to 22 mycotoxins can be detected (Gimeno 1979, 1980; Scott 1982b; Johann and Dose 1983; Nowotny et al. 1983). However, such methods are rarely quantitative and information on the type of fungal contamination of the commodity would assist in reducing the number of mycotoxins to be looked for.

Assays based on a biological test (bioassays) may be used for screening purposes and for confirmation of a mycotoxin detected by physiochemical means (Watson and Lindsay 1982). The chicken embryo assay has been employed in the past by the U.S. Food and Drug Administration for confirmation of toxicity of aflatoxin B_1, but has been replaced by a chemical test involving formation of derivatives of aflatoxins B_1 and G_1 on the TLC plate. Mass spectrometric confirmation of aflatoxin identity, particularly negative ion chemical ionization mass spectrometry, offers promise as a highly specific confirmation test for aflatoxins (Brumley et al. 1981; Nesheim and Brumley 1981).

7.3 ASSESSMENT OF QUANTITATIVE METHODS

Reference has been made above to sampling errors in mycotoxin determination and to interlaboratory collaborative study of the actual analytical methods. The analytical errors occurring within one laboratory for aflatoxin determination in raw peanuts by TLC have been shown to be largely due to the quantification step (coefficient of variation 18.6%) (Whitaker and Dickens 1981). Methods for aflatoxin analysis (at the 10 $\mu g.kg^{-1}$ order of contamination) that achieved coefficients of variation of 30-40% in interlaboratory collaborative studies (with recoveries of ≥70%) were considered eligible for referee status by Schuller et al. (1976). However, even larger coefficients of variation obtained in international check sample programs (laboratory proficiency studies, in fact) are a matter of some concern (Friesen et al. 1980; McKinney 1981). For example, for analysis of aflatoxin B_1 in peanut butter by the Contaminants Branch (CB) and Best Foods (BF) methods (AOAC Official Methods I and II) at the 4-6 $\mu g.kg^{-1}$ level, coefficients of variation were 72 and 101%, respectively, in one check sample series (Friesen et al. 1980). Clearly, some individual laboratory performance needs improvement, and training is called for. Also no method is better than the standards used in it, and recommended procedures for checking concentration and purity must be followed (Stoloff 1980).

It is important to use the right method for the commodity being analyzed since they are sometimes matrix dependent. For example, the BF method, which is valid for peanuts, gave much lower recoveries of aflatoxins than the CB method when applied to corn (Shotwell and Goulden 1977). The need for quality control when applying methods, i.e. recovery data on blank samples, is apparent.

Assessment of method performance for other mycotoxins is more limited than for the aflatoxins. Currently available quantitative methods for determination of trichothecenes were recently evaluated for accuracy, precision and limits of detection (Scott 1982a). A short list of the more accurate and sensitive GLC and radioimmunoassay methods was discussed further with respect to convenience and the number of trichothecenes analyzed. No collaborative studies have been carried out so far, but these are being planned for DON methods. It should be emphasized that a rapid method is needed for the determination of DON in wheat; the charcoal/alumina column method of Romer et al. (1981) appears promising. Although there are numerous methods available for zearalenone, another Fusarium mycotoxin that occurs naturally in Canadian grains (Bennett and Shotwell 1979; Cohen and Lapointe 1980; Scott 1981b; Trenholm et al. 1981a), only one has been collaboratively studied and interlaboratory performance of these methods remains to be evaluated accurately. There are two official AOAC methods for the determination of ochratoxin A and recently this mycotoxin has been included in an international check sample program (Friesen and Garren 1982). Nesheim (1979) and Stoloff (1982b) have critically discussed the extraction, cleanup and quantitation steps in aflatoxin analysis and similar principles apply to other mycotoxins.

7.4 MYCOTOXIN STANDARDS (J.C. Young)

Reference compounds are required in mycotoxin studies to (i) aid in the identification of known mycotoxins isolated from naturally or artificially contaminated materials and (ii) quantitate the levels of known mycotoxins in such samples. These compounds may also be used in the characterization of unknown mycotoxins by analogy with chemical, physical and biological properties. For mycotoxin studies, it is imperative that the samples of the individual mycotoxins used as standard reference materials be of the highest possible purity.

The first step, then, is to obtain reference mycotoxins. As an area of investigation becomes established (such as for aflatoxin, citrinin, ergot alkaloids, ochratoxin, patulin, and zearalenone, to name but a few), the mycotoxins become commercially available (see Anonymous 1982b for a list of mycotoxin suppliers). Because of the complex structures of most mycotoxins, they are usually prepared by biological rather than chemical means. As with most areas of commerce, caveat

emptor also applies to mycotoxin standards; occasionally the sample vial may contain as little as 20% of the substance named on the label. The discrepancy may be due to changes, unknown to the manufacturer, occurring in the biological processes (i.e. fungal culturing) or to chemical changes in the sample itself due to its inherent instability or external forces (e.g. thermal) during shipping or storage. For emerging areas, e.g. the trichothecenes, samples not commercially available may sometimes be obtained, usually in small (i.e. mg) amounts, by personal contact with the scientists working with the mycotoxins in question. If these avenues fail, then it may be necessary to produce the mycotoxin on one's own, usually by obtaining the appropriate fungal strain, growing it on a suitable medium, and then extracting and purifying the desired compound. Close cooperation between trained mycologists and chemists is essential for success.

Once the reference material is in hand, it is necessary to establish the purity of the material. This is usually accomplished by determining the chemical, physical, and occasionally, biological properties. In addition to melting point, specific rotation, and circular dichroism measurements, one commonly determines the spectral properties (such as infrared (IR), ultraviolet (UV), fluorescence, proton and carbon-13 nuclear magnetic resonance (NMR), and mass (MS), with the molar UV absorptivity and absence of additional absorption or emission bands used as criteria of purity) and chromatographic properties (such as TLC, GLC and HPLC with the absence of additional spots or peaks on the chromatogram taken as criteria of purity). Chemical properties include color reactions, such as on a TLC plate, and pre- or post-column derivatization for GC or HPLC. Confidence in assignment of purity increases when two or more independent properties are determined. Although many of these properties have been reported in the open literature, the use of different instrument models sometimes makes spectral comparisons difficult. Thus, reference to compilations of physicochemical data made under identical conditions (Dusold et al. 1978; Cole and Cox 1981; Pohland et al. 1982) is useful. Detailed comments on purity criteria for selected mycotoxin standard reference materials are given in reviews by the Association of Official Analytical Chemists (Stoloff 1980) and the International Agency for Research on Cancer (Anonymous 1982b).

Radiolabelled mycotoxins generally are not commercially available and except for the aflatoxins must be prepared either by chemical modification of "cold" material or by incorporation of labelled precursors via fungal culturing. Much research is still needed in this area.

7.5 CONCLUSION

In conclusion, analytical methods of differing degrees of reliability exist in profusion for known mycotoxins. They have enabled

surveys of Canadian foods for aflatoxins in nuts, nut products and other foods; ochratoxin A, zearalenone, DON, and ergot alkaloids in grains or grain foods; and patulin in apple juice; and have indicated the occurrence of tenuazonic acids in tomato paste and roquefortine in blue cheese. Methods have been specifically developed for these purposes when necessary. At present only aflatoxins and DON are subject to control in the Canadian food supply. Method development should continue in these areas and also to explore new potential problems. Further studies on sampling of commodities for mycotoxin analysis are required. Methods are needed or could be greatly improved for several mycotoxins that might occur in foodstuffs, e.g. cyclopiazonic acid, tremorgens, secalonic acids, xanthomegnin and related pigments, cytochalasins, rubratoxins, and macrocyclic trichothecenes.

8.0 DECONTAMINATION

(J.C. Young)

The preferred line of defense against mycotoxin contamination in food and feed is prevention. However, despite the best efforts of breeders to develop resistant plants, and of farmers to use clean seeds, good agronomic practices and fungicides when appropriate, and to have the crop stored under ideal conditions, fungal infection and concomitant mycotoxin formation still occur. In such cases, it may be possible to treat the product so that the mycotoxin is removed or destroyed.

The ideal decontamination method would be effective, inexpensive and simple. It would not create new toxins, not alter the nutritional and other desirable parameters of the food or feed, and it would use existing technology.

Of all mycotoxins, aflatoxin has been investigated in greatest detail, with some success being achieved with practically every technique. Although aflatoxin has been rarely reported in native Canadian commodities, there is concern over its possible importation in some nut products. Whether it will become a problem in the burgeoning peanut industry in this country remains to be seen. Doyle et al. (1982) have extensively reviewed decontamination methods for aflatoxin, patulin, and rubratoxin in foods and feeds.

8.1 PHYSICAL METHODS

Fungi that produce well defined sclerotia, such as ergot in cereals, can sometimes be removed by sieving or by flotation in a brine solution (Seaman 1980). The latter method unfortunately yields damp, salty seeds that must be further treated.

Some mycotoxins are thermally unstable and can be destroyed by heating. Following the observation that pelleting, which generates heat, reduced the toxicity of ergot-contaminated feed (Friend and Macintyre 1969; Bragg et al. 1970), subsequent studies showed that autoclaving or heating by convection or microwaves reduced the ergot alkaloid content of sclerotia and also reduced their toxicity to chickens (Young et al. 1983b; Rotter et al. 1984). Only 5% of the aflatoxin in peanuts (Luter et al. 1982) and half the vomitoxin in corn (Young et al. 1984b) remained after microwave heating to 150°C.

Irradiation with ultraviolet light or X- or Y-rays shows more promise in controlling fungal growth than in destroying mycotoxins.

It may be possible to remove some mycotoxins from aqueous solutions by extraction with suitable organic solvents.

Mycotoxins have been adsorbed from solution with solids such as activated charcoal and the clay bentonite (Doyle et al. 1982). A proprietary polyvinylpyrrolidone mixture is claimed (Sagmeister 1981) to adsorb mycotoxins from contaminated animal feeds once ingested by the animal; it has been shown to be effective in reducing the toxicity of some contaminated feeds (Halma 1982) but ineffective for others (Friend et al. 1984; Rotter et al. 1984). Alfalfa, a fibre-rich feedstuff, has been shown to be effective in reducing zearalenone toxicity in rats and swine (Smith 1980a, b) although there appear to be species differences in the protective effect mechanisms, such as adsorption or altered metabolism (James and Smith 1982). Zeolite, an anion-exchange resin, has also been shown to have a protective effect against zearalenone toxicity (Smith 1980a, b). Dietary manipulation in the prevention and treatment of mycotoxins has already been discussed in Section 5.3.

Milling hard red spring wheat contaminated with DON (4.6 $\mu g.g^{-1}$) and baking flour into bread did not have any significant effect on the mycotoxin level (El-Banna et al. 1983; Scott et al. 1983). On the other hand, milling contaminated soft white winter wheat yielded a fractionation of vomitoxin; increased levels were found in the bran and middling fractions, whereas the various flour fractions showed reduced levels (Young et al. 1984a). When these flours were baked into cookies and cakes, further reductions were obtained. The baking of bread and pancakes from a variety of ergot contaminated flours afforded alkaloid losses of up to 100% (Scott and Lawrence 1982). During the wet milling of corn, 66% of T-2 toxin was removed in the steep and process water (Collins and Rosen 1981).

Although not a decontamination method per se, dilution is widely used to reduce the toxicity of contaminated feeds.

8.2 CHEMICAL METHODS

Possible chemical methods of decontamination include the use of gases such as ammonia, chlorine, ethylene oxide, hydrogen chloride, ozone and sulfur dioxide. Ammonia has found extensive use in the decontamination of aflatoxin contaminated feedstuffs (Norred 1982) and is effective in reducing vomitoxin levels in contaminated corn (Young et al. 1984b) and in barley naturally contaminated with ochratoxin A (Madsen et al. 1983). The bleaching agents chlorine and ozone greatly reduced DON levels in contaminated corn (Young et al. 1984b). Chlorine is effective in reducing the alkaloid level of ergot sclerotia (Young et al. 1983a; Rotter et al. 1984).

Liquid treatments, such as with acids, alkali, bleach, peroxide and bisulfite have been reviewed by Doyle et al. (1982). Bennett et al. (1981) reported significant reduction of zearalenone levels when fermentation residues were treated with formaldehyde.

Food additives can help reduce mycotoxin levels in processed foods. Matsuura et al. (1981) observed that when ammonium peroxydisulfate or potassium carbonate was added to cake flour, the rate of thermal decomposition of zearalenone was enhanced about fivefold. Similarly, the levels of vomitoxin were further reduced when ammonium carbonate was added to samples of contaminated corn (Young et al. 1984b) and wheat (Young et al. 1984a) prior to their being heated.

8.3 BIOLOGICAL METHODS

A variety of enzymes and microorganisms can degrade mycotoxins, particularly in liquid samples (Doyle et al. 1982). When a commodity is so heavily contaminated that it is unfit for use as a food or feed, it may have to be diverted to another end use such as a feedstock for ethanol production by fermentation. Bennett et al. (1981) observed that zearalenone was not destroyed when contaminated corn was fermented, although the ethanol produced was free of the mycotoxin; zearalenone was concentrated in the residual solids and solubles, which are generally used for animal feed.

9.0 MYCOTOXINS AS A NATIONAL SECURITY ISSUE

(H.B. Schiefer)

The debate over the alleged use of mycotoxins ("yellow rain") as chemical warfare (CW) agents in Laos, Kampuchea and Afghanistan (Ember 1984) has now developed into a discussion beyond the straightforward question of whether the trichothecenes were used (Casselli 1983) and has become significant in terms of the development of criteria that are needed to investigate allegations of such use.

Chemical warfare, in all its guises, holds a special significance for Canadians. When the first massive military use of chemical weapons was initiated in 1915 at Ypres, Canadian soldiers were amongst the first victims of this new type of warfare. The event produced a psychological effect on generations of Canadians and, in part at least, accounts for Canada's seeming preoccupation with this issue, despite the fact that the Geneva Protocol of 1925 prohibits "the use of any poisonous, asphyxiating or other gases and all analogous liquids, materials and devices," and the 1972 Biological Weapons Convention forbids its parties from "development, production, stockpiling, acquisition or retention of microbial or other biological agents or toxins, whatever their origin of production." The only real difference between the two treaties is that the 1972 Biological Weapons Convention provides for verification procedures. Verification is probably the single most significant issue in the Arms Control and Disarmament process in the decade of the 1980's. Before one can verify a violation, one has to have methods to prove that a CW or biological warfare (BW) agent has indeed been used. At present, false allegations cannot be disproven; true allegations cannot be verified, particularly if the agent in question happens to be a substance which may occur naturally.

With regard to national security aspects of mycotoxins in case of terrorist actions or threats in Canada it is appropriate to consider the following points specifically. Toxic effects due to trichothecenes can be induced through the alimentary, respiratory and topical routes. In order to cause effects via the alimentary canal, a certain amount of the toxin has to be ingested. It is highly unlikely that enough toxin could be introduced via the food chain or water supply system, when one considers the average North American diet consists of a variety of commodities from different sources. Further, the burning sensation that occurs in the mouth would probably prohibit voluntary intake of contaminated food after a first taste. Little is known about the respiratory route, because there are no published experimental studies. Yet, given an appropriate method of aerosol production, it is conceivable that humans exposed to sprays or mists directly, might suffer. Houses and other protective measures (e.g. gas masks, etc.) would probably reduce the total possible amounts to be inhaled. Similarly, the uptake of effective amounts of toxin by the tropical

route would be highly unlikely if several layers of clothing are worn for much of the year. Specific treatments or prophylactic measures await investigation (Anonymous 1983).

10.0 GAPS IN KNOWLEDGE AND RECOMMENDATIONS FOR RESEARCH IDENTIFIED BY THE PANEL OF AUTHORS

(Compiled by H.L. Trenholm and P.M. Scott)

10.1 IMPACT ON AGRICULTURE

10.1.1 PROBLEM DEFINITION

There is very limited information on the extent and nature of actual disease outbreaks in Canada that can be associated with mycotoxin contamination. In 1980, when DON was detected in the spring and winter wheat crops in eastern Canada, extensive surveys were carried out on the extent of DON contamination of Canadian grain crops. However, less survey research has been carried out on other potentially hazardous known, as well as currently unknown, mycotoxins. Several laboratories across Canada are analyzing mycotoxins in suspected feed and grain samples, but little work is underway in the form of well-designed surveys to define the nature and extent of mycotoxins in feeds and foodstuffs. Molds capable of producing various mycotoxins are known to be present in Canadian feedstuffs. For example, Penicillium cyclopium, which produces penicillic acid, has been isolated from Canadian grains and feeds and penicillic acid has been reported in corn, beans and tobacco crops in the United States. However, little information is available on the penicillic acid content of Canadian crops.

The reports of investigators at several Canadian centers who have documented outbreaks of animal problems in which mycotoxins were suspected probably represent just the tip of the iceberg. Less still is known about the problems due to subtle effects of low dietary levels of mycotoxins (alone or in combination with any other associated causal factors, e.g. nutritional status, stress) on livestock and poultry production.

The development and implementation of a national registry program, involving standardized protocols for collecting information on outbreaks when mycotoxin contamination is suspected in animal feedstuffs, and standard methods of chemical analysis for feedstuffs, blood and urine would, over a period of years, greatly enhance our data base on mycotoxin-related problems. The information would give us new and better insights into the incidence, nature and causal factors involved in livestock and poultry mycotoxicoses.

10.1.2 MOLD INFECTION AND MYCOTOXIN PRODUCTION IN PLANTS AND PLANT PRODUCTS

Much research in needed on molds infecting and producing toxins in field crops and plant products. More work is necessary on the prevention of mold infestation and subsequent production of mycotoxins through studies of the life cycles of fungi, farm management

practices, development of plant resistance and use of pesticides, and other means to control mold invasion and mycotoxin production.

In more specific areas of research, techniques need to be developed for more efficient inoculation methods that simulate natural infections in order to develop effective screening methods for resistant plant genotypes. There is a need to study the occurrence and development of mycotoxins in storage, including ecological parameters affecting mycotoxin production. An evaluation is needed of potential toxin production in stored seeds of present and future cultivars of certain crops as a means of reducing hazards.

10.1.3 ANIMAL PRODUCTIVITY AND HEALTH

More research is needed on the effects of mycotoxins on Canadian livestock and poultry production. DON and zearalenone are just two Fusarium metabolites. There are reports of outbreaks of toxicosis in farm animals, but the levels of DON and other mycotoxins in these suspected feeds are often lower that those known to cause effects in feeding trials carried out at research establishments. One must conclude that the animals involved in such reported field outbreaks were either much more sensitive than the animals used in feeding experiments, or that other factors, e.g. poor sampling, inaccurate chemical analyses, nutritional and environmental stresses on the animals, poor health, or other fungal metabolites not yet identified, were involved.

Feeding trials with livestock and poultry need to be conducted to determine dose-response relationships for mycotoxins and the mechanism(s) of action by which mycotoxins affect livestock and poultry. Comparative studies among livestock and poultry species are needed to determine the relative tolerance of different species and types to various mycotoxins. The results of the research would lead to better preventive measures and effective means to treat and reduce acute and chronic mycotoxicoses in livestock and poultry. Information relating to species, sex, age or type of animal or bird able to tolerate diets containing mycotoxins, would then be available.

Studies need to be carried out on the effects of ingesting mycotoxin combinations on production performance, since there are reports of the presence of several mycotoxins in suspected feeds. Interactions with other toxins, nutritional and environmental parameters and health status need investigation to resolve these very important and complex issues. Priority should also be given to research that addresses the problems of whether mycotoxins predispose animals to secondary infections through immunosuppressve action and how the toxins are absorbed, metabolized and excreted.

Once livestock or poultry species have been identified as being tolerant to particular mycotoxin(s), the concern is redirected

from the mycotoxin effects on productive performance to mycotoxin residues in animal products for human consumption, e.g. milk, eggs, meat. Research is needed on mycotoxin residues and the toxicity of metabolites in animal products.

Research must include the development of practical techniques for decontamination or reduction of the potency of mycotoxins in animal feeds. Approaches that need to be tested include detoxification by physicochemical means or milling operations, addition of toxin absorbing materials to diets, or the use of nutritional feed additives that reduce the harmful effects or aid detoxification by farm animals.

As part of the animal research, there is an urgent need to develop bioassay techniques to test for small quantities of mycotoxins in suspected feed samples.

10.2 IMPACT ON HUMAN HEALTH

10.2.1 INTAKE THROUGH DIET

There is little knowledge of the chronic toxicity or carcinogenicity for several of the mycotoxins known or suspected to occur in the Canadian food supply, e.g. DON, Alternaria mycotoxins and ergot alkaloids. In addition, there are conflicting reports on the teratogenic potential of ergot alkaloids.

Intake data can be established only by surveys of Canadian foods for particular mycotoxins. Some extrapolation from mycotoxin concentrations and incidence in field crops can be made if the effects of milling and food processing are known.

As was the case with DON in 1980, there are still many important toxins e.g. macrocyclic trichothecenes, tenuazonic acid and other Alternaria toxins and cyclopiazonic acid for which a good understanding of the health hazards is lacking.

10.2.2 INTAKE VIA INHALATION

Mycotoxins can pose a particular hazard to those involved in handling contaminated crops, e.g. personnel involved in harvesting, processing and distributing contaminated crops. Information is required as to which mycotoxins are present in grain dust, as to the levels of exposure (from inhalation of dust and fungal spores) for those working with contaminated crops and food commodities. Occupational exposure to aflatoxin, if any, in the Canadian food and feed processing industry (e.g. those handling contaminated Brazil nuts) also needs to be assessed. While much information is lacking on the oral route of exposure, that is ingestion of contaminated feeds or foods, even less is

known about human exposure to mycotoxins through the dermal route or through inhalation. Methods to prevent exposure to mycotoxins by these latter routes need to be developed.

10.3 TOXICOLOGY

Our understanding of the pharmacology and toxicology of mycotoxins in still in its infancy. In the case of certain toxins, e.g. aflatoxin, T-2 toxin, ochratoxin A and perhaps some of the ergot alkaloids, there is a body of scientific literature, but it is far from complete. Only during the past few years have we had any meaningful information on other mycotoxins, e.g. zearalenone and DON. It was not until DON was detected in eastern Canadian wheat that a major research program was initiated to determine safe levels in feed and food commodities. Even then, toxicology studies progressed slowly until scientists in other disciplines were able to develop sensitive analytical methods and pure chemicals for animal testing.

The research cannot be restricted to DON, zearalenone and other known and still unknown Fusarium toxins. Studies on the toxicity of other fungal metabolites suspected to be in Canadian grains and feed and food commodities need to be initiated. The objective of the research would be to have a clear understanding of the toxicology of mycotoxins of importance to agriculture and food industries.

Limited studies have been carried out with pure mycotoxins. Without such studies there can be no basic framework within which to draw comparisons with feeding trials involving naturally contaminated diets. Although some toxicological research has been carried out on trichothecenes such as T-2 toxin and DON, there is little information available on macrocyclic trichothecenes which may, in fact, be more toxic.

Finally, it should be stressed that much of the research on the toxicology of mycotoxins is dependent on sensitive analytical methods and on the availability of pure mycotoxins and radiolabelled metabolites. Without the support of research by scientists in the other disciplines, progress on the toxicology of mycotoxins will be slow and arduous.

10.4 MYCOLOGICAL RESEARCH

Apart from studies being carried out at the Atlantic Research Laboratory, NRCC, on toxins from fungi collected in Nova Scotia (Section 1.12 and 1.13); at the Ottawa Laboratories, Agriculture Canada, on trichothecenes from Fusarium (Section 1.15); and at the London Research Centre, Agriculture Canada, on Alternaria polyketides, little is being done to study biosynthesis and to find new mycotoxins or other metabolites produced by Canadian fungal isolates and posing a potential

health hazard for animals or man. A systematic examination of such isolates for new and known mycotoxins, not only from grains, but also from other agricultural commodities destined for food or feed use would be a mammoth undertaking. Studies are needed on the occurrence, distribution and taxonomy of toxigenic fusaria in Canada with particular emphasis on their capacity to produce toxins in plant foodstuffs and feedstuffs in which they are likely to occur. At present we do not even know, for example, whether the trichothecene nivalenol is produced by Canadian strains of Fusarium graminearum. T-2 toxin and diacetoxyscirpenol (DAS) have been reported to occur naturally in Canada and Fusarium species that produce these toxins have been identified.

As part of our need to acquire information on what toxigenic fungi are involved, agreement needs to be reached on a universally accepted taxonomic system for Fusarium species. Taxonomists need to resolve existing differences in species concepts, to understand and document the broad range of morphological and physiological differences of fusaria, and to establish internationally acceptable reference collections of cultures.

More information is needed on other species. The toxigenic potential of Alternaria and Chaetomium isolates reported on a wide variety of substrates in Canada needs to be carefully assessed. Reports of isocyanide metabolites from a number of species of Trichoderma and the role that these metabolites may play in the ill-thrift syndrome in sheep clearly indicate that more research is needed on the etiology of Trichoderma in soil and its relationship to mycotoxin problems in livestock production.

10.5 ANALYTICAL METHODS

Quantitative and qualitative analyses of mycotoxins constitute a most critical reference point not only in research and surveillance, but also in regulatory work. Lack of adequate analytical support has been a continuing bottleneck in our understanding of the safety of mold-contaminated grains for use in animal feeds and food products. When DON was first identified in the 1980 white winter wheat in eastern Canada, rapid analytical methods, and supplies of standards of pure DON were not available for survey work to define the extent and seriousness of the problem or for feeding trials to evaluate the toxicology of DON in experimental and farm animals.

More specifically, studies need to be carried out to establish the appropriate sampling protocols for wheat and other small grains with respect to analyses for DON and other mycotoxins of importance to Canada. The determination of an appropriate minimum sample size is essential for meaningful surveys of the nature and extent of mycotoxin contamination. A rapid and reliable method for the determination of DON

in wheat and other grains is required. Methods are also required or need to be greatly improved for the determination of several other mycotoxins with potential for occurrence in Canadian foods and feeds, e.g. cyclopiazonic acid, secalonic acids and xanthomegnin and related pigments. Research is needed on the production and purity criteria of mycotoxin reference standards and radiolabelled mycotoxins. Radiolabelled mycotoxins, of which few are commercially available, are required at adequate specific activities[α] to study their metabolism in animals and assess residues in milk, eggs and edible animal tissues. Reliable methods for analyses of blood, urine and tissues to detect mycotoxins or their metabolites are needed to provide evidence in cases of animal mycotoxicoses and to examine residues in animal products. Currently, analytical methods are not readily available to detect many mycotoxins and major metabolites at the trace levels where they are affecting nutritional and health parameters. Further development of multi-mycotoxin analyses for all types of matrices is necessary in order to provide more information on mycotoxin occurrence. However, there is a practical limit to the number of mycotoxins that can be included and it is necessary to be selective in deciding for which mycotoxins we really do need to establish occurrence data.

10.6 SUMMARY

In a world where mission-oriented research is supreme, a major problem is that we do not have a clear definition of the nature and extent of mycotoxins present in Canadian feeds and food commodities, other than those already investigated after a problem manifested itself (e.g. aflatoxin and DON). Likewise, we do not have a comprehensive picture of what mycotoxins are responsible for the serious outbreaks and of what are the more subtle long-term effects of mycotoxicoses in Canadian livestock and poultry.

Without a clear definition of the seriousness of the problems, it is difficult to direct limited scientific resources to address priority national issues of feed and food safety aspects of mycotoxins. Exceptions were the bringing under control in the 1960's and 1970's of the main possible source of aflatoxins in the Canadian diet, peanut butter, and the multi-disciplinary effort of Canadian researchers to address the urgent problem of DON contamination of eastern Canadian grain crops. These were both "fire fighting" operations to meet a possible perceived crisis of confidence in a food or feed supply. In the latter case, once the fungus and the mycotoxin were identified, a comprehensive research program was initiated to develop suitable

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[α] specific activity: total radioactivity of a given radioisotope per molecular weight of a compound: now expressed in becquerel.mole^{-1} (formerly in curies.mole^{-1} or millicuries.mole^{-1}).

analytical methods and pure standards, to prepare pure material for toxicology studies, to carry out studies to prevent Fusarium infections and toxin production in the field, to study the toxicology in experimental animals, livestock and poultry, and to develop and test effective means of detoxification. Major efforts were initiated which involved close collaboration between many research establishments in Canada and abroad culminating, in the case of aflatoxin, only 20 years later in our present more or less complete control of the problem without yet possessing an understanding of the epidemiology in humans; in the case of DON all the necessary research has not yet been done.

The above examples of aflatoxin in food and DON contamination are presented to illustrate what needs to be done with other important mycotoxin contaminants in Canada before they turn out to cause a crisis situation. The major Canadian mycotoxin problems, in terms of extent and nature (levels of mycotoxins in Canadian grains, feed and food commodities and problems observed in livestock and poultry), need to be defined in order to focus sufficient resources on the multi-disciplinary, mission-oriented research with the much broader view-point required to forestall such crises.

APPENDIX I

GUIDELINES FOR THE SAFE HANDLING OF MYCOTOXINS IN RESEARCH LABORATORIES[α]

H.L. Trenholm and J.C. Young

By definition, mycotoxins and the fungi that produce them are health hazards. The broad range of research carried out on mycotoxins requires the development of guidelines that will cover all present and future situations. In the interim the following recommended practices are advised. They stress several basic concepts, namely:

The responsibility for implementing safety guidelines rests with the principal scientist involved in a particular experiment, although all staff must be safety conscious. Everyone has a personal responsibility to be aware of the health hazards associated with the mycotoxin(s) under investigation and to use laboratory practices that emphasize accident prevention and minimize contamination of laboratory facilities and personnel.

Every reasonable precaution must be taken to minimize mycotoxin exposure by any route, e.g. oral, inhalation, cutaneous and injection.

An ongoing program of monitoring laboratory procedures and levels of laboratory contamination must be carried out on a regular basis.

Laboratory procedures need to be in place for routine handling and disposal of contaminated material, and for emergencies and accidents.

Medical examinations of all laboratory workers engaged in mycotoxin research should be carried out annually or more frequently if excessive exposure warrants it.

The following guidelines are recommended for the safe handling of mycotoxins in a research laboratory. It is essential, however, that protective equipment must be used in a responsible manner. Accidents may still occur - e.g. Bamburg and Strong (1971) describe an incident in which an extract of T-2 toxin in ethylacetate accidentally got inside plastic gloves worn by two laboratory workers, resulting in severe dermatitis.

A. When handling mycotoxins and toxigenic fungi or contaminated samples and feedstuffs:

α A.R.C. Contribution No. 1276.

1. Avoid exposure to toxic vapors, mists and dusts by preventing the escape of toxic material into the working environment, and ensure adequate ventilation, e.g. exhaust hoods and keeping toxic materials downwind in field experiments. Perform all procedures carefully to minimize the formation of aerosols and dusts. Pure mycotoxins in the dry state may readily become electrostatically charged and dispersed into the air and surrounding working area. Where possible, prepare a stock solution for research purposes to reduce the hazards of working with crystalline toxins. When handling pure solid mycotoxins, disposable cotton gloves are preferable to latex gloves to reduce electrostatic dispersion of the mycotoxins as solid dust.

2. Avoid direct contact and minimize skin contact and inhalation by wearing protective equipment including gloves, coveralls or lab coats, surgical masks and goggles.

3. Use mechanical pipetting devices; pipetting by mouth should be prohibited.

4. Store pure supplies in double breakproof containers in locked storage with only minimum working quantities of mycotoxins in any work area.

5. Work areas where mycotoxins are being handled must be clearly identified as hazard areas. Do not eat, drink, smoke, store food, or apply cosmetics in the work area.

6. Post the entrances to all work and storage areas where carcinogenic mycotoxins (e.g. aflatoxin) are present with CANCER HAZARD warning signs. Where carcinogen producing fungi are present, post entrances with BIOHAZARD warning signs.

7. Ensure that work areas are properly ventilated, e.g. with fume hoods and exhaust fans. Dust inhalation must be avoided by the use of fume hoods or surgical masks. If mycotoxins or fungal contaminants are present in exhaust aerosols and particulate matter, use effective microparticulate (e.g. HEPA) filters or scrubbers for protection during maintenance operations.

8. Wash hands and face and any exposed skin upon completion of work with mycotoxins.

9. Use work surfaces that are impervious to water and resistant to acids, alkalis, and organic solvents. The use of plastic-backed absorbent towelling over work surfaces facilitates cleanup and minimizes aerosols from spills.

10. Regularly clean all equipment and bench tops with decontaminating agents.

B. When preparing and feeding contaminated diets, in addition to the above:

1. Utilize when possible, diet formulations that minimize dust particulates and aerosols (e.g. use diets with oil or molasses bases). Alternatively, if the mycotoxin is very toxic, it should be administered as a bolus, by gavage or in capsule form to protect personnel.

2. If possible, do not keep other animals in the same immediate work area as the animals being used for mycotoxin experiments.

3. Clean mixers well after use to prevent contamination of other diets.

4. Clean feeders and pens before and after feeding trials involving mycotoxins and pesticides.

5. Brief all operational staff preparing, handling or feeding contaminated feed on the hazards through a responsible scientist, technician or supervisor.

C. Cleaning and decontamination:

1. The work area must be arranged so that it may be easily cleaned.

2. Equipment, working surfaces and exhaust systems must be cleaned/decontaminated singly or sequentially, as necessary, with detergents, bleaching agents, or decontaminating agents.

3. All heavily contaminated liquid or solid wastes must be decontaminated or destroyed by incineration under the direct supervision of the principal scientist before being disposed of or otherwise handled.

4. Contaminated materials that are to be decontaminated at a site away from the work area must be placed in a durable leakproof container which is closed before being removed from the work area. The container should be clearly marked to indicate what mycotoxin is present, its health hazard and the name of the responsible scientist.

D. Mycotoxins, contaminated feed and feedstuffs must be stored in well-labelled containers in a locked area separate from other samples and feedstuffs. Labels should clearly indicate special hazards, e.g., CARCINOGEN, TOXIC, RADIOLABELLED, BIOHAZARD.

E. All employees working with mycotoxins and toxigenic fungi, must receive periodic medical examinations. Any suspicious symptoms must be reported immediately to the supervisor and nurse.

F. All spills and accidents must be reported immediately to the supervisor. In the event of a major accident involving heavily contaminated material, the chairman of the Joint Occupational Safety and Health committee, or equivalent, must also be advised.

G. A copy of these guidelines should be prominently posted in work areas where mycotoxin research is being carried out (e.g. laboratories, feed mill, feed storage area and where animal experiments are done).

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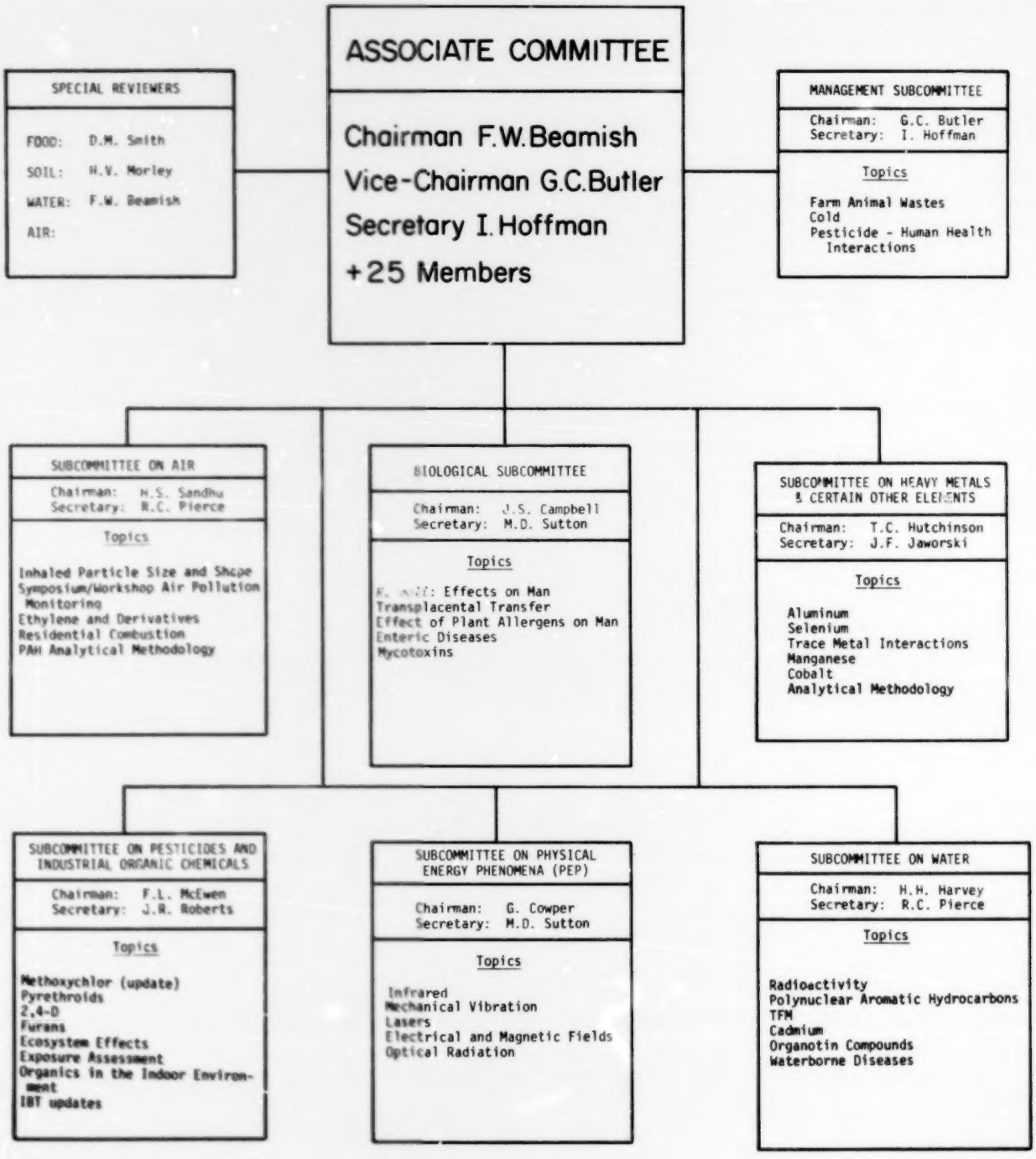
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